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EVOLUTIONARY TENDENCIES AMONG GYMNOSPERMS

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The investigation of gymnosperms has proceeded with such vigor that some adequate picture of the group freed from its details may now be obtained. The confusion of details often obscures the important facts, and it may be of service, at this stage of our knowledge, to emphasize them. There is no better way in which to develop a clear picture of a great group than to select those facts of structure that enter into its general evolutionary history. This has nothing to do with differences among species, or even among genera, which may be left to the taxonomist; but it deals with those general tendencies to change structures which can be noted in passing from the most ancient gymnosperms to the most recent.

While the discovery of these tendencies aids in reaching conclusions in reference to the phylogenetic connections of the groups of gymnosperms, it must be remembered that the tendencies are facts and the phylogenetic conclusions are very uncertain inferences. Moreover, a general tendency expresses itself throughout a great group, and has to do with the transition from ancient to modern forms, rather than with the breaking-up of the group into several phylogenetic lines. Failure to remember this fact has been responsible for much sterile inference as to relationships, similar stages in some general tendency being assumed to mean immediate genetic connection. The organism is a plexus of structures, and must be considered in its totality when relationships are being considered. Among the general tendencies leading to the origin of seed plants, for example, that which resulted in heterospory must be regarded as of paramount importance;

and yet it is clear that heterospory arose several times, and probably many times, independently as the natural result of a general tendency among pteridophytes. To put into the same genetic group all heterosporous pteridophytes would be regarded now as a morphological absurdity; and yet there have been repeated suggestions of relationship, especially among conifers, on the basis of certain features of the female gametophyte, for example, features which represent a stage in a general change that may occur in a number of independent lines.

In passing from the ancient to the modern gymnosperms, it becomes evident that groups differ as to the rapidity with which they respond to a general tendency to change, and it, is this difference that helps to constitute groups. A modern group, for example, may associate a number of ancient features with others that are recent; or all of the ancient features may have been changed. This has been called the "lagging behind" of certain structures, but it should not imply that they are held back and will come forward later; it simply means that for some reason they have not responded to the general tendency among gymnosperms to change in a certain direction. The retention of an old structure must not be confused with the reappearance of an old structure. For example, it seems clear that the most ancient gymnosperms were large-leaved forms, from which the small-leaved conifers were derived; and yet small-leaved pteridophytes were probably more ancient than large-leaved ones. If this be true, the appearance of small leaves among conifers is the reappearance of an ancient feature, and not its retention. To prove the retention of an ancient feature demands the establishment of its phylogenetic continuity.

PHYLOGENY

Before sketching the general evolutionary tendencies among gymnosperms, it will be of service to outline what seems to be a reasonable conclusion as to the phylogenetic connections of the groups. This statement and those that follow will be all the more clear if freed from the details upon which they are based, and also from citations. Those who know the facts and the investigators do not need their recital; and those who do not know them would only be confused by their recital.

The Paleozoic groups Cycadofilicales[1] and Cordaitales represent the historical background of gymnosperms. They are of equal age, so far as the records are available, and are so connected by intergrading (or rather anastomosing) forms that their relationship seems evident. The Cycadofilicales are so fern-like in every feature except their seeds, that their derivation from some ancient fern stock (called provisionally Primofilices) is as certain as phylogenetic connections can be. The origin of the Cordaitales, therefore, presents two alternatives: either they arose independently from the same ancient fern stock, or they were differentiated from the Cycadofilicales very early. To choose between these alternatives is not very important, but the latter one seems to be the more reasonable, because the Cordaitales (as we know them) are much more removed from the ferns than are the Cycadofilicales. If this conclusion is accepted, it follows that gymnosperms began with Cycadofilicales more ancient than any yet known; that Cordaitales branched off from Cycadofilicales earlier than our present records; and that the two groups constituted the extensive gymnosperm flora of the Carboniferous.

This Paleozoic display of gymnosperms was succeeded by a Mesozoic display, in which at least four groups are recognized. From the Cycadofilicales there arose the Mesozoic Bennettitales and the Cycadales; and from the Cordaitales the Mesozoic Ginkgoales and Coniferales were derived. The Bennettitales have been traced almost to the Paleozoic, and their structure, as well as the habit of some of the earlier forms, make their connection with the Cycadofilicales appear convincing. The relation of the Bennettitales to the Cycadales is not so clear; either the two groups were differentiated from a common stock that arose from the Cycadofilicales and continued into the Mesozoic, or the Cycadales were differentiated early from the Bennettitales. The records show that the Cycadales are much younger

[1] It seems impossible at present to recognize a group Pteridosperms as distinct from Cycadofilices, and there is a growing impression that the two groups are coextensive. Under the circumstances, it would seem proper to retain for the group the older name. It seems clear, also, that it should take its place as a group of gymnosperms coordinate with Cordaitales, Cycadales, Coniferales, etc.; for it is impossible as yet to construct a definition which would exclude it from gymnosperms on the basis of characters of so high an order as those which separate gymnosperms from angiosperms. For this reason, I have appended to the older name the termination that makes it conform to the names of groups of similar rank, and shall refer to it in this paper as the CYCADOFILICALES.

than the Bennettitales, and were much more scantily represented during the Mesozoic; and therefore the latter alternative seems to be the more reasonable. In any event, the only question at issue is whether the gymnosperm stock which came from the Cycadofilicales into the Mesozoic is to be called Bennettitales or a Bennettitales-Cycadales plexus (a "common stock"); and it is altogether probable that this stock has already been assigned to the Bennettitales in the description of cycadophyte forms from the Triassic and Jurassic. In the gymnosperm flora of today, therefore, the Cycadales, although relatively a modern group, are the nearest representatives of the Paleozoic Cycadofilicales.

The Ginkgoales and Coniferales have both been traced into late and independent Paleozoic connection with the Cordaitales, and were well displayed during the Mesozoic. The Ginkgoales, while widely distributed during the Mesozoic, apparently were never a large group; and this group has continued as a single line into the present flora, and has retained certain features of the Cordaitales which the Coniferales have lost. The Coniferales, on the other hand, began that extensive differentiation during the Mesozoic which has resulted in six recognized tribes in our present flora. Among these tribes the earliest to be recognized are the Abietineae and the Araucarineae, and their very early separation is so evident as to raise the question whether they may not be independent in origin. In any event, the other tribes recognized in our present flora were of later origin; the Taxodineae and the Cupressineae, and possibly the Taxineae, arising from the Mesozoic Abietineae; and the Podocarpineae possibly arising from the Mesozoic Araucarineae.

The connections of the Gnetales are altogether obscure, and every opinion as to their origin must be regarded as very tentative. Although they have not been discovered as fossils, the great amount of differentiation they show and their widely scattered geographical distribution indicate a considerable history. Evidence seems to be accumulating that they may have been derived from the Cupressineae, or at least that they are closely related to that tribe in origin.

VASCULAR ANATOMY

The central cylinder of the Cycadofilicales, like that of ferns, was protostelic, siphonostelic, or polystelic. Whatever may be the

genetic connection of these types of cylinder, the siphonostele is the type that was carried forward in the evolution of gymnosperms. This siphonostele was made up of collateral mesarch bundles, developed secondary wood composed of tracheids, and the bundles of all its peripheral connections were concentric or at least mesarch.

Among the gymnosperms the universal tendency was to eliminate the centripetal xylem, a tendency carried forward from the ferns, until the collateral mesarch bundles of the central cylinder became collateral endarch; and more gradually the bundles of the peripheral regions became collateral mesarch and finally collateral endarch. So early was this accomplished for the central cylinder that a collateral endarch cylinder is a feature of gymnosperms in general. From what has been said as to the variable rate of change among the members of a great group, it would be expected that mesarch and even concentric bundles might be found in peripheral parts of certain species of genera whose allies had completely eliminated centripetal xylem, or might occur occasionally in any species or genus. It is an interesting fact that centripetal xylem appears to linger longest in the cotyledons; and the number of gymnosperms in which it is known to occur in this organ, regularly or occasionally, is increasing rapidly.

In the development of secondary wood, the general tendency among gymnosperms is to increase it in amount, so that a thick vascular cylinder is built up by the primary cambium. This tendency is apparent among the Cordaitales, but it reaches its most conspicuous result in the Coniferales and Ginkgoales. This changed also the general topography of the stem, both pith and cortex being much reduced in relative amount. The Bennettitales and Cycadales responded feebly if at all to this tendency, one of their features being the retention of the general stem structure of the Cycadofilicales. In these groups the primary cambium is either short-lived or functions very slowly, and in some forms secondary cambium produces cortical bundles; but the formation of secondary wood never prevents the formation of a large pith and an extensive cortex. It is in these groups also that the concentric and mesarch bundles are most common in the peripheral members, being found somewhere in all forms; while among the Coniferales the centripetal xylem has been

almost completely eliminated. In vascular anatomy therefore, the Cycadales have retained more ancient features than any other living group.

The vascular condition among Gnetales can hardly be spoken of in connection with general tendencies; but the appearance of true vessels associated with the tracheids of the secondary wood is too important to omit. In any event, these true vessels of the secondary wood suggest that in the evolution of the vascular cylinder the original tracheids of secondary wood are finally and gradually replaced by true vessels.

THE LEAF

The leaves of gymnosperms may be used to illustrate a structure that exhibits no general evolutionary tendency, but responds more or less directly to the conditions of living. The most ancient gymnosperms possessed ample, fern-like leaves, and under appropriate conditions this type of leaf persisted, as in the tropical cycads of today. The conifers, however, have developed a very different type of leaf, one that was well under way among the Cordaitales, and which reaches an extreme expression in small and rigid needles or concrescent scales. This cannot be regarded as the result of a general tendency among gymnosperms, quite unrelated to conditions of living, such as is shown by the persistent progressive changes of other structures. The leaf is too variable a structure, and too closely related in its work to external conditions to permit such an explanation of its changes.

It would be interesting to know the conditions in which needles and concrescent disks were established; but in the absence of any such knowledge, the sharply contrasted geographical distribution of Cycadales and Coniferales may suggest that the conditions of change were associated with the evolution of the land areas and of the climate of the temperate regions.

THE STROBILUS

The Cycadofilicales are the only gymnosperms without strobili. Although the sporophylls differ more or less from the fern-like foliage leaves or their branches, they are not aggregated into a strobilus distinct from the rest of the shoot. The organization of a strobilus

by the shortening of the sporophyll-bearing shoot is a conspicuous feature of gymnosperms, and it must have been derived from the condition observed among the Cycadofilicales.

The Cordaitales were the first gymnosperms to produce strobili, and this is one of their conspicuous contrasts with Cycadofilicales. The record of the structure of their strobili is meager, but it shows several tendencies in strobilus-formation. Of primary importance is the fact that the strobili are monosporangiate, and this monosporangiate character prevails throughout the Ginkgoales and Coniferales. Among the Gnetales, a group probably related to the general coniferophyte phylum, amphisporangiate strobili occur in Tumboa. If this connection be accepted, therefore, these amphisporangiate strobili have been derived from monosporangiate strobili. It is not necessary to associate in one genetic connection all of the amphisporangiate gymnosperms, for that condition doubtless appeared independently several times, just as the monosporangiate strobilus is known to have appeared at least twice in distinct phyla (Cordaitales and Cycadophytes).

Another fact in reference to the strobili of Cordaitales, which must stand for the most ancient gymnosperm strobili, is that they included both simple and compound strobili. The staminate strobilus was simple, that is, its sporophylls were borne directly upon the axis of the strobilus; and this type of staminate strobilus persisted throughout the Ginkgoales and Coniferales. Among the Gnetales the staminate strobilus is compound, the individual simple strobilus being borne on axes of the second order in the axils of sterile bracts which make up the general strobilus. There is an evident relationship between the compact compound staminate strobilus, such as occurs in Ephedra and in Tumboa, and the short foliage branch bearing axillary simple staminate strobili, as in Torreya. Even in Gnetum the compound staminate strobilus is a loose one; and among the taxads there is a tendency to compact the staminate strobiliferous branch. The conclusion is that the staminate strobilus among coniferophytes is quite persistently simple, but that in the more modern members of the phylum it tends to become compound, a condition accomplished by compacting a short strobiliferous shoot.

The ovulate strobilus of Cordaitales was compound, at least in

the very few specimens sectioned; that is, the ovules were borne on short secondary and bractlet-bearing axes that arose in the axils of the sterile and overlapping bracts that constituted the strobilus. This compound ovulate strobilus is a distinctive feature of the coniferophytes, prevailing among the Pinaceae and characterizing the Gnetales. That simple ovulate strobili may have been derived from it is quite possible. For example, in Torreya the ovulate strobili are simple and are axillary on short leafy branches, just such a branch as could have arisen through the elongation of the axis of a compound ovulate strobilus, so that the sterile bracts could be replaced by foliage leaves. It may be said that the change may have taken place in the other direction, and that the short leafy strobiliferous branch was compacted into a compound ovulate strobilus; but it must be remembered that the Cordaitales with their compound ovulate strobili are very old, and that the Taxineae with their leafy strobiliferous branches are relatively very recent. Of course it may be discovered that the Cordaitales included also forms with simple strobili on leafy shoots. This possibility is further emphasized by the fact that the ovulate strobili of the Araucarineae, and of their allies the Podocarpineae, are simple. The former tribe is a very old one, and its connection with the Cordaitales is either direct or nearly so, so that it is altogether probable that such ovulate strobili occurred in that group. The connection of the Taxineae with the Cordaitales, however, appears to be so remote, and their relation to groups with compound ovulate strobili seems to be so much more immediate, that it is more reasonable to suppose that their ovulate strobiliferous branches have arisen from compound strobili in the way described above.

Long after the Cordaitales had established their simple staminate and compound ovulate strobili, strobili appeared in the cycadophyte phylum, being found in Bennettitales and Cycadales; and even in the living Cycas the loose ovulate strobilus retains the evidence of its origin from the separated sporophylls of Cycadofilicales. The cycadophyte strobilus has always been simple, and this may be related to the more compact habit of body, with its lack of free-branching. The most remarkable feature of the early strobili of this phylum, however, is their amphisporangiate character, the two sets of sporo-

phylls holding the same relation to one another that is held by the stamens and carpels of angiosperms; and this marks the Bennettitales as a unique group among gymnosperms. The monosporangiate tendency, however, which characterizes the coniferophytes, is shown by the Cycadales among cycadophytes, and it was either established directly, or it arose from an early differentiation of the amphisporangiate strobilus of the Bennettitales. The evidence of history favors the latter view, but the probabilities of the situation favor the former. In any event, both monosporangiate and amphisporangiate strobili were established among cycadophytes.

THE STAMEN

Among gymnosperms the stamen may be regarded as a very conservative structure, retaining throughout most of the phyla its fern-like characteristics. Its form perhaps became almost as much differentiated among the Cycadofilicales as it ever became among gymnosperms. In this primitive group, in addition to microsporophylls resembling fern-like leaves with abaxial synangia, there appeared others that have been spoken of as the Crossotheca ("epaulet") and the Calymmatotheca ("cupule") types, and in all probability still others will be discovered. All of these types were continued among the coniferophytes, with varying details of minor importance. Among some of the Gnetales, either the sporophyll has become very much reduced, or it has become suppressed, so that the microsporangia are cauline; but even in Tumboa the old terminal synangium is evident.

Among the cycadophytes, on the other hand, only what may be regarded as the most ancient type of microsporophyll has been retained, that is, the fern-type with abaxial sporangia (often synangia). Among the Bennettitales, there is so little departure from the old type that their microsporophylls resemble pinnate fern leaves with abaxial synangia; and even among the Cycadales the more or less leaf-like microsporophylls show the same character. If there is any tendency in the stamens of this phylum worth noting, it is the tendency shown among the cycads to reduce the sterile apex of the sporophyll to a more compact peltate expansion.

The microsporangium of gymnosperms is a very consistent struc-

ture, originating from the hypodermal layer of cells, and developing a wall of several layers, the innermost one of which is usually differentiated as the tapetum. The only general tendencies to be observed are the gradual replacement of synangia by separate sporangia, and the more rapid elimination of all evidences of an annulus (in the general sense). It is noteworthy that in both these particulars the cycadophytes, with their much more recent connection with the Cycadofilicales, are far behind the coniferophytes.

THE OVULE

The origin of the ovule of gymnosperms remains in obscurity. While the stamen and its sporangia repeat the corresponding structures of ferns, the ovules of Cycadofilicales and of Cordaitales are so well organized, even in the modern sense, that their connection with the sporangia or synangia of ferns is entirely a matter of inference. This means a tremendous gap between the somewhat hypothetical Primofilices, on the one hand, and the Cycadofilicales and Cordaitales, as we know them, on the other hand, a gap which there seems to be small probability of filling up with intermediate forms. In this presentation the only thing possible is to take the ovules of the investigated Paleozoic forms as representing the oldest known ovules, and to note the general changes that have occurred since.

To select the most primitive type of ovule from among the Paleozoic forms that have been investigated is impossible, unless it is assumed that those ovules which are most unlike the modern ones represent the most primitive type. This may or may not be true, but it is the only available criterion; and by using it, we obtain the following result. The oldest ovule had a single integument entirely free from the nucellus; in testa-formation this integument differentiated into three layers, the outer fleshy, the stony, and the inner fleshy; the ovule was supplied with two sets of vascular strands, the outer set traversing the outer fleshy layer, and the inner set traversing the peripheral region of the nucellus; and the beaked tip of the nucellus broke down more or less completely within the firm and resistant epidermis to form a pollen chamber. If these are really the features of the most primitive known ovules, the changes become very apparent, and they represent general tendencies, for they appear in every phylum.

In the first place, the integument and nucellus, instead of remaining separate, develop separately only in the region of the nucellar beak. So early was this change that it probably represents the condition of the majority of the Paleozoic ovules, a condition which has persisted ever since. The method of development is very evident, the integument appearing first as a distinct annular growth about the base of the young nucellus, but later its basal meristematic zone becoming indistinguishable from that of the nucellus. In all probability the change was brought about by the earlier appearance of the integument, and the result has been more or less variability in the amount of freedom from the nucellus.

The three-layered testa persists remarkably throughout gymnosperms, varying chiefly in the amount of development of the outer fleshy layer. The stony layer is always strongly developed, and at the maturity of the seed the inner fleshy layer always forms for it a papery lining. A strong development of the outer fleshy layer, resulting in fleshy seeds, continues throughout the cycadophyte phylum and the Ginkgoales and is a feature of many of the Taxaceae. Among the Pinaceae the outer fleshy layer is present in the young integument, but does not develop, so that the stony layer is the conspicuous superficial feature of the seed. The development of the outer fleshy layer among the Cycadales and Ginkgoales is phylogenetically continuous from the Cycadofilicales and Cordaitales; but among the Taxaceae there is probably no such continuity, but a reappearance of the activity of this layer in certain genera. Among the Gnetales, the single integument of the other gymnosperms is replaced by two integuments, the inner fleshy layer having become differentiated as a delicate inner integument, which appears earlier than the heavier outer integument, which gives rise to the outer fleshy and stony layers. In Gnetum the outer fleshy layer develops the pulpy investment characteristic of the primitive seeds; but in Ephedra this layer behaves as among the Pinaceae. If any general tendencies can be inferred from these facts in reference to the integument and testa they are seen in the abortion of the outer fleshy layer in the largest group of living gymnosperms, and in the final differentiation of the three-layered integument into two integuments.

The vascular supply of the ovule exhibits a very evident progressive

change. When the integument and nucellus become free only in the region of the nucellar beak, the inner set of vascular strands is shifted from the peripheral region of the nucellus to the inner fleshy layer, and this situation persists among the cycadophytes. Curiously enough, it reappears in Gnetum, but in that case it is associated with the presence of the two integuments. Among Ginkgoales the outer set of strands is suppressed; among Taxaceae the inner set is suppressed; and among Pinaceae both sets have disappeared. The general tendency, therefore, is to eliminate the vascular strands from the ovule; but it is puzzling to find both sets absent from the older Abietineae, and one set still present among the younger Taxineae.

The presence of a pollen chamber is one of the most conspicuous features of the primitive ovule, and its association with fertilization by ciliated sperms is so evident that it is natural that the two disappeared simultaneously with the establishment of Coniferales, but the abruptness of the disappearance is evidently more apparent than real. The presence of an extraordinarily deep pollen chamber in Ephedra can hardly be regarded as a contradiction to this general statement, for in that case it is evidently of secondary origin, associated with a remarkably massive archegonium neck.

THE FEMALE GAMETOPHYTE

The female gametophyte of gymnosperms exhibits a progressive series of changes which is significant because it leads toward the angiosperm condition. At this point the very important historical record fails, and the entire testimony must be obtained from living forms, which do not represent a series, but the ends of many series. For this reason, and also because such progress is always very unequal in different forms, various stages of advancement may be expected to be found in forms grouped in a single alliance. The series, therefore, is not so much one which conforms to recognized groups, as a series of stages each of which may be exhibited by members of various groups.

The general development of the gametophyte is quite uniform; and since the same sequence of events occurs among the heterosporous pteridophytes, it may be inferred that a knowledge of the ancient

gymnosperms would not materially change this situation. The general sequence referred to in the development of the gametophyte is as follows: free nuclear division, usually accompanied by vacuolation which results at some stage in parietal placing; the formation of walls, resulting in a parietal tissue; the centripetal growth of this tissue until it reaches the center of the embryo sac; and the final growth of the gametophyte until it reaches its mature size. In certain cases there may be no parietal placing, the free nuclei remaining distributed throughout the embryo sac; and therefore there is no centripetal growth, but general wall-formation throughout the sac. The details of the formation of permanent endosperm tissue from the primary walled cells are variable and perhaps very important from the evolutionary point of view, but the range of forms from which these details have been obtained is far too small to make them of present service in this connection.

The tendency which runs through gymnosperms as a whole, and which reaches its extreme expression among angiosperms, is to mature the eggs earlier and earlier in the ontogeny of the gametophyte. In the most primitive condition of the gametophyte, the eggs do not appear until the endosperm is nearly full grown; and other gametophytes can be selected and arranged in a series showing the gradual slipping-back of the egg in the ontogeny of the gametophyte, until in such a form as Torreya the archegonium initial is differentiated as soon as wall-formation has taken place. A conspicuous illustration of the inequality of response to such a general tendency among related forms is furnished by Torreya and Cephalotaxus, the archegonia not appearing in the latter genus until the gametophyte is well grown. The next stage is illustrated by the situation in Tumboa, in which eggs are matured before wall-formation is complete, resulting in the elimination of archegonia. The extreme stage in this progressive series of changes among gymnosperms is illustrated by Gnetum, in which eggs are matured at the stage of free nuclear division, the most embryonic stage of the female gametophyte.

So far as the living forms are concerned, the Cycadales and Ginkgoales show little, if any, response to this tendency; and therefore possess the most primitive type of female gametophyte among living gymnosperms. Among the Coniferales, on the other hand,

all stages are found, to the one just preceding the elimination of archegonia; and this stage is attained by Tumboa and Gnetum.

The general tendency of the archegonia among gymnosperms is to eliminate the ventral canal cell. The gymnosperms are distinguished from the pteridophytes by the complete elimination of neck canal cells, and this tendency to suppress all of the axial row except the egg continues among gymnosperms. Among the living forms, a walled ventral canal cell is retained only among the Abietineae, so far as known; but it seems to be a safe inference that it was present among the ancient gymnosperms. In the other living groups the wall has disappeared, and the ventral canal cell is represented by a free nucleus. In certain forms even this nucleus may have disappeared; and of course there is no trace of it when the archegonia are eliminated.

The distribution of archegonia may be considered in this connection, although the tendencies do not appear general. It is becoming evident that the position of archegonia is related to the position of the pollen tube, which often reaches the embryo sac before the archegonium initials are selected. In cases where the pollen tube assumes a lateral position in reference to the gametophyte (as in Sequoia and Widdringtonia), it has been demonstrated that the latter responds by the selection of numerous deep-seated and laterally placed archegonium initials. It may be inferred, therefore, that the usual micropylar position of archegonia is due to the usual micropylar position of the tip of the pollen tube. It may be that numerous scattered and rather indefinitely placed archegonia were a feature of ancient gymnosperms, but there is no evidence for it; on the contrary, the few sections of Paleozoic ovules that reveal archegonia, and also the archegonia of heterosporous pteridophytes, suggest the opposite conclusion. In any event, they tend to become definite in number and are then organized in two ways: either as individual archegonia, each with its own jacket and chamber; or as an archegonial complex, with a common jacket and chamber. The latter may seem to be a specialized condition, exhibited chiefly by Cupressineae, but it also seems to be the natural condition from which to derive the free eggs of Tumboa and Gnetum when archegonia are eliminated from ontogeny.

THE MALE GAMETOPHYTE

It is perhaps impossible as yet to determine the character of the male gametophyte of the Paleozoic gymnosperms. The evidence is accumulating that it comprised many more cells than do the gametophytes of most living gymnosperms; but it is not demonstrable whether these supernumerary cells were vegetative or spermatogenous. There are instances of supernumerary cells of both kinds among living gymnosperms, so that they furnish no clue; and the same is true of heterosporous pteridophytes. The balance of probability, however, is in favor of the view that they were in the main spermatogenous.

In any event, starting with the known condition among heterosporous pteridophytes, the tendency among gymnosperms has been to reduce and finally to eliminate the vegetative (prothallial) tissue; and to reduce the sperm mother cells to two.

In certain groups (as Abietineae) the prothallial cells are two in number; in others (as cycads) there is one prothallial cell; and in still others (as Taxodineae, Cupressineae, and Taxineae) prothallial tissue has been eliminated. Such prothallial cells as do appear are sometimes persistent and sometimes ephemeral; so that the evidence of a disappearing tissue is complete, and it actually has disappeared in what are recognized as the most modern groups. The situation common to Podocarpineae and Araucarineae is usually cited as an illustration of a more extensive and therefore a more ancient prothallial tissue, which connects directly with the "multicellular" pollen grains of Cordaitales. This may be true, but all of the extra cells are derived from two primary ones, which hold a definite place in the ontogeny of the gametophyte; and therefore may represent a secondary tissue that holds no phylogenetic relation to the more extensive prothallial tissues of older forms. In any event, it is ephemeral, breaking down and liberating its nuclei.

The number of sperm mother cells is so rigidly two, that this reduction may be said to have been accomplished by all living gymnosperms, whatever may be the fact in reference to the Paleozoic gymnosperms. It is interesting to note that the very few instances of a greater number of sperm mother cells occur in one group characterized by its retention of ancient features (Cycadales), and in another

group characterized by its very modern features (Cupressineae). While phylogenetic continuity of multiple sperms might be claimed for Cycadales, no such claim could be maintained for Cupressineae.

The greatest epoch in the history of the male gametophyte of gymnosperms, however, was the abandonment of ciliated sperms, and this occurred in connection with the establishment of Coniferales. It is not generally appreciated that five of the seven recognized primary groups of gymnosperms possess ciliated sperms, and that the modern type or completely terrestrial type of sperm was introduced by the conifers. This also affected the pollen chamber, the pollen tube, and the cell generations in spermatogenesis. The pollen chamber disappeared; the pollen tube ceased to be exclusively a branching haustorial organ and became a sperm-carrier; while the last cell-generation in spermatogenesis was omitted. It is this condition of spermatogenesis that is carried forward by angiosperms to a still greater stage of reduction. Just what cells are eliminated and what cells remain is a question of small importance. The significant fact is that spermatogenesis is shortened, and the ultimate cells, although non-ciliated, are physiologically sperms.

THE EMBRYO

The absence of embryos from the seeds of Paleozoic gymnosperms indicates that some great change connected with embryo-formation was introduced by the Mesozoic gymnosperms. It would be of extreme interest to know the ancient condition, but we know only what it has become. After this change, whatever it may have been, the proembryo has become the structure showing steady and progressive change.

The first stage in the development of the proembryo is free nuclear division, followed by wall-formation; and in the most primitive condition the free nuclei are so numerous that wall-formation results in a tissue which fills the egg. The tendency is to reduce the number of free nuclear divisions, resulting in a reduction of the amount of proembryonic tissue, so that more and more of the general cytoplasm of the egg is left free from tissue. The proembryo of Ginkgo has retained a very primitive character; and illustrations of early stages in the reduction of the proembryo may be observed among the cycads.

In Zamia, for example, which illustrates the extreme amount of reduction among cycads, the proembryo is a relatively small amount of tissue at the base of the egg. Among the Coniferales this reduction has been carried forward to a much greater extent, 16 free nuclei appearing sometimes, 8 free nuclei being the prevailing number, and 4 free nuclei being occasionally attained. This results in a proembryo consisting of a small and definite number of cells, with distinctly distributed functions. Now and then among gymnosperms (as in Sequoia), there is no free nuclear division, but these are interesting rather than significant exceptions. Even among the Gnetales it is found that a certain amount of free nuclear division precedes wall-formation. That this reduction of the free nuclei before wall-formation is significant, is shown by the fact that among angiosperms free nuclei have disappeared, and wall-formation accompanies the first division of the egg-nucleus.

The University of Chicago

ON SIMILARITY IN THE BEHAVIOR OF SODIUM AND POTASSIUM

W. J. V. Osterhout

(WITH FOUR FIGURES)

It is commonly mentioned by textbooks, as worthy of remark, that sodium and potassium agree closely in chemical behavior, but differ fundamentally in their effects upon plants.

This general statement is founded on the study of the nutritive functions of sodium and potassium. There is no a-priori reason for supposing it to be true in the field of toxic or of protective action. As this is a point of general interest I have made some experiments with reference to it.

Two extensive series of experiments, one on sodium, the other on potassium, were carried on simultaneously. They were found to show a remarkable degree of agreement in the action of these two substances.

The experiments relating to sodium have already been described,[1] while those on potassium have been withheld from publication, pending thecompletion of further observations on the mutually antagonistic action of sodium and potassium.

Most of the experiments were made with a variety of wheat known as Early Genesee. The technique has been fully described in a previous paper.[2]

TOXIC ACTION

In the earliest studies which I made on balanced solutions, I was struck with the fact that Na and K agree closely in their toxic effect on plants.

These results I have found to hold in an extensive series of experiments, including algae, liverworts, Equisetum, and some thirteen genera of flowering plants. While there are doubtless some exceptions, the general rule seems to be that Na and K are closely similar in their toxic action.

[1] Jahrb. Wiss. Bot. **46**:121. 1908.

[2] Bot. Gazette **44**:266. 1907.

The most careful quantitative studies which we possess on this point are those of Miss MAGOWAN.[3] These studies show that the toxicity curves for K and Na are practically identical, while the corresponding curve for Mg shows a much higher, and that for Ca a much lower degree of toxicity.

ANTAGONISTIC ACTION[4]

The antagonistic action of monovalent kations on each other has especial interest in view of the experiments of LOEB[5] on Fundulus, which offer a certain analogy with those of LINDER and PICTON.[6] In these experiments monovalent kations antagonized bivalent but did not antagonize other monovalent kations.

The curve of antagonism between NaCl and KCl shows two maxima. The location of these maxima, however, is not constant, but varies somewhat in different series of experiments. Table I and *fig. 1* show the average result of four series. The antagonism

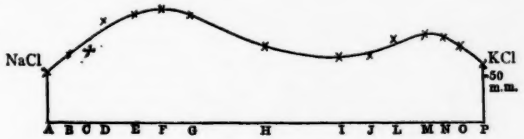

FIG. 1.—Antagonism curve, NaCl vs. KCl. The ordinates represent millimeters of growth of the roots of wheat. The ordinate at A represents the growth in pure NaCl, that at P the growth in pure KCl. The other ordinates represent growth in mixtures of NaCl and KCl, the proportions of which are found opposite the corresponding letters in table I: thus the ordinate at H represents growth in a mixture of 100^{cc} NaCl + 100^{cc} KCl.

is comparatively slight. I have also noticed antagonism between Na and K in liverworts.

Table II and *fig. 2* show that both Na and K antagonize NH_4, and that their effects are very similar.

[3] *Ibid.*, **45**:45. 1908.

[4] Cf. facts and literature given by KEARNEY and CAMERON, Rept. No. 71, U. S. Dept. Agr. 1902; and by BENECKE, Ber. Deutsch. Bot. Gesells. **25**:322. 1907.

[5] American Journal of Physiology **3**:327. 1900.

[6] Cf. HOBER UND GORDON, Hofmeister's Beitr. Chem. Physiol. und Pathol. **5**:432. 1904.

TABLE I

WHEAT (GROWTH DURING 30 DAYS). ALL QUANTITIES GIVEN ARE CUBIC CENTIMETERS OF 0.12 *m* SOLUTIONS

Culture solution	Corresponding point on curve (*fig. 1*)	Aggregate length of roots per plant in mm
NaCl	A	55
100 NaCl / 5 KCl	B	75
100 NaCl / 10 KCl	C	80
100 NaCl / 15 KCl	D	115
100 NaCl / 25 KCl	E	120
100 NaCl / 35 KCl	F	130
100 NaCl / 50 KCl	G	121
100 NaCl / 100 KCl	H	85
50 NaCl / 100 KCl	I	75
35 NaCl / 100 KCl	J	80
25 NaCl / 100 KCl	L	95
15 NaCl / 100 KCl	M	100
10 NaCl / 100 KCl	N	95
5 NaCl / 100 KCl	O	85
KCl	P	65

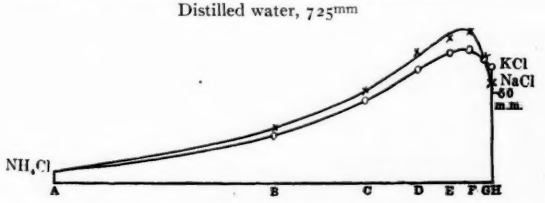

FIG. 2.—Antagonism curve, NH_4Cl vs. NaCl (upper curve -x-x-x-) and NH_4Cl vs. KCl (lower curve -o-o-o-). Each ordinate represents the amount of growth of wheat roots in a solution whose composition is given opposite the corresponding letter in table II.

TABLE II

WHEAT (GROWTH DURING 30 DAYS). ALL QUANTITIES GIVEN ARE CUBIC CENTIMETERS OF 0.12 *m* SOLUTIONS

Culture solution	Aggregate length of roots per plant in mm	Corresponding point on curve (*fig. 2*)	Culture solution	Aggregate length of roots per plant in mm
NHC_4l.....	6.2	A	NH_4Cl.....	6.2
100 NH_4Cl } 100 NaCl }	31	B	100 NH_4Cl } 100 KCl }	27.5
40 NH_4Cl } 100 NaCl }	52	C	40 NH_4Cl } 100 KCl }	46
20 NH_4Cl } 100 NaCl }	74	D	20 NH_4Cl } 100 KCl }	62
10 NH_4Cl } 100 NaCl }	80	E	10 NH_4Cl } 100 KCl }	72.5
5 NH_4Cl } 100 NaCl }	85	F	5 NH_4Cl } 100 KCl }	75
1 NH_4Cl } 100 NaCl }	67	G	1 NH_4Cl } 100 KCl }	68
NaCl.....	55	H	KCl.....	66

Distilled water, 725 mm

Experiments with magnesium show that it is antagonized in about the same degree by both Na and K (table III and *fig. 3*).

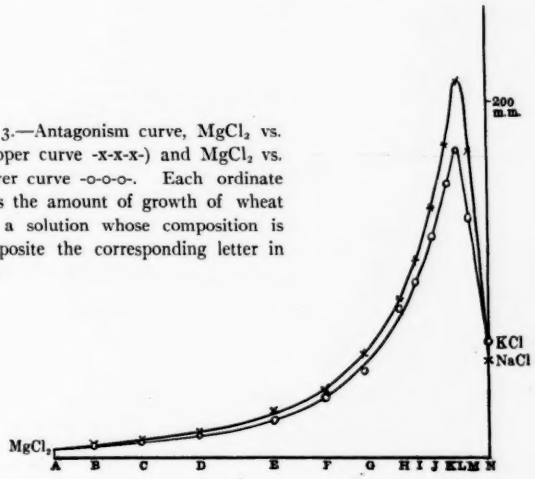

FIG. 3.—Antagonism curve, $MgCl_2$ vs. NaCl (upper curve -x-x-x-) and $MgCl_2$ vs. KCl (lower curve -o-o-o-. Each ordinate represents the amount of growth of wheat roots in a solution whose composition is given opposite the corresponding letter in table III.

TABLE III

WHEAT (GROWTH DURING 30 DAYS). ALL QUANTITIES GIVEN ARE CUBIC CENTIMETERS OF 0.12 *m* SOLUTIONS

Culture solution	Aggregate length of roots per plant in mm	Corresponding point on curve (*fig. 3*)	Culture solution	Aggregate length of roots per plant in mm
$MgCl_2$	5	A	$MgCl_2$	5
100 $MgCl_2$ } 10 NaCl }	7.5	B	100 $MgCl_2$ } 10 KCl }	7.5
100 $MgCl_2$ } 25 NaCl }	10	C	100 $MgCl_2$ } 25 KCl }	8.7
100 $MgCl_2$ } 50 NaCl }	13.7	D	100 $MgCl_2$ } 50 KCl }	12.5
100 $MgCl_2$ } 100 NaCl }	25	E	100 $MgCl_2$ } 100 KCl }	21
60 $MgCl_2$ } 100 NaCl }	37.5	F	60 $MgCl_2$ } 100 KCl }	34
40 $MgCl_2$ } 100 NaCl }	50.7	G	40 $MgCl_2$ } 100 KCl }	48.7
25 $MgCl_2$ } 100 NaCl }	90	H	25 $MgCl_2$ } 100 KCl }	84
20 $MgCl_2$ } 100 NaCl }	112	I	20 $MgCl_2$ } 100 KCl }	99
15 $MgCl_2$ } 100 NaCl }	140	J	15 $MgCl_2$ } 100 KCl }	125
10 $MgCl_2$ } 100 NaCl }	176	K	10 $MgCl_2$ } 100 KCl }	152
7.5 $MgCl_2$ } 100 NaCl }	210	L	7.5 $MgCl_2$ } 100 KCl }	171
5 $MgCl_2$ } 100 NaCl }	172	M	5 $MgCl_2$ } 100 KCl }	135
NaCl.....	55	N	NaCl.....	66

Distilled water, 725 mm

In algae I have found that $MgCl_2$ is much more strikingly antagonized by KCl than by NaCl.

The experiments with calcium show a more marked antagonism than any of the other cases. We find that Ca is antagonized to a slightly greater degree by K than by Na (table IV and *fig. 4*).

Similar results were obtained with algae, liverworts, Equisetum, and some fifteen genera of flowering plants.

From the facts here set forth it is clear that in their toxic and

protective effects sodium and potassium show great similarity. As this does not seem to be the case in the field of nutritive effects, we

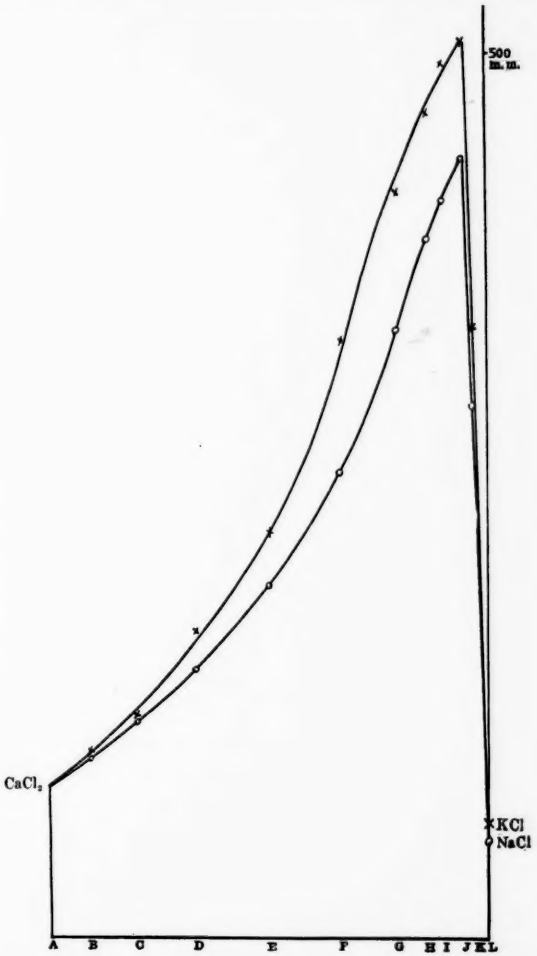

FIG. 4.—Antagonism curve, $CaCl_2$ vs. KCl (upper curve -x-x-x-) and $CaCl_2$ vs. NaCl (lower curve o-o-o). Each ordinate represents the amount of growth of wheat roots in a solution whose composition is given opposite the corresponding letter in table IV

seem to have in this case a means of distinguishing clearly between nutritive and protective action.

TABLE IV

WHEAT (GROWTH DURING 30 DAYS). ALL QUANTITIES GIVEN ARE CUBIC CENTIMETERS OF 0.12 *m* SOLUTIONS

Culture solution	Aggregate length of roots per plant in mm	Corresponding point on curve (*fig. 4*)	Culture solution	Aggregate length of roots per plant in mm
$CaCl_2$	85	A	$CaCl_2$	85
100 $CaCl_2$ + 10 NaCl	100	B	100 $CaCl_2$ + 10 KCl	105
100 $CaCl_2$ + 25 NaCl	117	C	100 $CaCl_2$ + 25 KCl	125
100 $CaCl_2$ + 50 NaCl	150	D	100 $CaCl_2$ + 50 KCl	174
100 $CaCl_2$ + 100 NaCl	198	E	100 $CaCl_2$ + 100 KCl	230
50 $CaCl_2$ + 100 NaCl	262	F	50 $CaCl_2$ + 100 KCl	337
25 $CaCl_2$ + 100 NaCl	342	G	25 $CaCl_2$ + 100 KCl	420
15 $CaCl_2$ + 100 NaCl	390	H	15 $CaCl_2$ + 100 KCl	464
10 $CaCl_2$ + 100 NaCl	416	I	10 $CaCl_2$ + 100 KCl	492
5 $CaCl_2$ + 100 NaCl	440	J	5 $CaCl_2$ + 100 KCl	507
1 $CaCl_2$ + 100 NaCl	300	K	1 $CaCl_2$ + 100 KCl	346
NaCl.....	55	L	KCl.....	66

Distilled water, 7.25 mm

SUMMARY

The accepted idea that sodium and potassium have entirely different effects upon plants is not valid in the field of toxic and protective action. Here their behavior shows the close similarity which their near chemical relationship would lead us to expect.

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TOXIC AND ANTAGONISTIC EFFECTS OF SALTS AS RELATED TO AMMONIFICATION BY *BACILLUS SUBTILIS*

CHAS. B. LIPMAN

(WITH FIVE FIGURES)

In the science of bacteriology, especially in that of soil bacteriology, our work has thus far only taught us enough to give added zest to investigation and the most important and interesting results are still forthcoming. RAMANN (**23**) well expresses it when he says, "In spite of numerous and important investigations we are still but in the first stages of our researches on bacteria and only at the very starting-point in our knowledge of soil bacteria." In his haste to classify bacteria, to show their direct relation to many diseases, to apply his knowledge of them to the practical side of the dairy industry, and finally, to measure the net results of the activities of bacteria in the soil, the bacteriologist has left almost untouched one of the most important phases of the science of bacteriology, namely, the physiology of bacteria, which is of great scientific interest and practical importance. Especially is this true of the physiology of soil bacteria, which remains as yet a closed book, and since the writer is devoting his time to researches on soil bacteria in particular, it was thought best at first to experiment with some of these organisms. It may also be added that in California, with its thousands of acres of waste alkali land, and in similar regions elsewhere this study will undoubtedly prove to be of the greatest practical significance, especially when we have learned to coordinate the results obtained in similar investigations on the higher plants with those derived from researches on soil bacteria.

Since ammonification is the first great step in the transformation and simplification of the organic soil nitrogen, it was thought best to study the effect of various salt solutions on pure cultures of ammonifiers first. The work was carried out along the same general lines as to the preparation of solutions as the experiments of LOEB on the effects of salt solutions on various forms of animal life, and the subse-

quent experiments of OSTERHOUT on the higher plants. It was indeed the insight of the latter investigator which led him to conclude some time ago that a proper understanding of physiologically balanced solutions in relation to plants and to soil bacteria would render the control and successful cultivation of alkali lands a much simpler matter than it has been, and this will undoubtedly prove true in the near future.

In the selection of an ammonifier which could be used uniformly throughout all the experiments, the writer was guided by the work of MARCHAL (**15**), to whom indeed we owe what little knowledge we have of the physiology of ammonifiers. Among the best ammonifiers found by that investigator were *B. mycoides*, which changed 46 per cent. of nitrogen into ammonia in a given time, *Proteus vulgaris* (36 per cent.), *Sarcina lutea* (27 per cent.), and *B. subtilis* (19 per cent). Since the last form is easily isolated and cultivated and is a strong ammonifier, it was decided to use it in the following experiments. It is more than probable that the same relative results will be obtained with any ammonifier; this, however, will be tested in other experiments now contemplated by the writer. The pure culture of *B. subtilis* employed for inoculation through all the series of experiments was obtained from soil from Auburn in the foothill fruit region of California.

The salts tested were the chlorids of sodium, potassium, calcium, and magnesium. Only chemically pure salts were used, after submitting them to a flame test. Molecular or bimolecular stock solutions in distilled water were made, from which the requisite amounts were taken for the various concentrations. Witte's peptone was the nitrogenous substance used for the ammonification, of which 1 per cent. solutions were employed in the tests for the single salts and 0.75 per cent. in the binary solutions. The method of inoculation employed was as follows: Inoculation is made from peptone agar slope of *B. subtilis* into a sterile 100^{cc} portion of 1 per cent. peptone in a 250^{cc} Erlenmeyer flask. This is incubated for forty-eight hours at 28° C., at the end of which time the membrane that forms on the surface of the culture is precipitated by slight shaking, and then by tilting the flask to one side and carefully setting it down again, the liquid covering part of the bottom of the flask remains free from membranous

material and is homogeneous in character. Of this homogeneous liquid 1^{cc} was drawn off with a sterile pipette for inoculation into each flask to be tested, the greatest caution being used to prevent any particle of membrane from entering the pipette as the liquid was drawn up. This was the most satisfactory method of inoculation of several tested and yields concordant results in the duplicate series. All the solutions employed were made practically neutral. The incubation was carried out in a thermostat at a temperature which varied between 28° and 29° C. The incubation period was two days in the case of the single salt solutions, and two and one-half days for the binary solutions. The amount of ammonia formed, which was used as a criterion for establishing the efficiency of *B. subtilis* in the various solutions, was determined as follows: At the end of the incubation period the culture solutions were transferred to flat-bottomed Jena distillation flasks, diluted to 300–350^{cc}, an excess of magnesium oxid added, and distilled. The amount of ammonia in the distillate was titrated against standard acid, cochineal being used as the indicator. Sterile blanks were run on all determinations, each of which was made in duplicate, and the tables given below represent averages of at least three sets of such duplicates and in some instances of five and six sets of duplicates.

Experiments with single salts

In determining the salts to be tested the writer was guided by the alkali and alkaline earth constituents of soils. The sodium, potassium, calcium, and magnesium salts are important factors in plant nutrition and are always present in soils; in some cases, indeed, one or more of them may be present in such excess as to inhibit plant growth materially and in some instances completely. Such soils we find in California and other states under the common appellation of "alkali lands." It was decided, therefore, to test the salts of the alkalies and alkaline earths above mentioned, to determine the degree of toxicity of each for the bacteria experimented upon. Since from similar work on animals and plants, the anion of salts was found to have comparatively little effect, a chlorid of each metal was employed for the sake of uniformity.

SERIES I. SODIUM CHLORID

There were prepared sixteen Erlenmeyer flasks (125^{cc} capacity), each containing 50^{cc} of solution, made up as follows: The first flask contained 50^{cc} of 1 per cent. peptone solution in distilled water; the second contained a 0.1 *m* solution of NaCl and 1 per cent. peptone; the third flask contained 0.2 *m* NaCl and 1 per cent. peptone; and so on, the concentration of NaCl increasing by 0.1 *m* in each succeeding flask up to the sixteenth, which contained 1.5 *m* NaCl and 1 per cent. peptone. All the flasks were plugged with cotton, sterilized in the autoclave at a pressure of 1.25 atmospheres, and when cool were inoculated from a culture of *B. subtilis* in the manner above described. After two days' incubation at 28° to 29° C., the ammonia formed in the peptone solutions was distilled off and determined as above explained. Table I shows the results.

TABLE I

Numbers represent tenths *m* NaCl solution	Milligrams of N formed as NH_3
0	5.60
1	6.79
2	4.13
3	2.80
4	2.59
5	2.45
6	2.38
7	2.10
8	1.89
9	1.54
10	1.33
11	1.05
12	0.91
13	0.28
14	0.28
15	0.28

Plotting a curve by laying off the concentrations as abscissae and the milligrams of N (as NH_3) as ordinates (*fig. 1*), we note the following: NaCl up to a concentration of 0.1 *m* stimulates *B. subtilis* as an ammonifier, but beyond that concentration it becomes gradually more and more toxic, and at 1.3 *m* we find scarcely any ammonification. It is evident, also, that NaCl is not nearly as toxic for *B. subtilis* as it has been found by LOEB (**8**, p. 412)[1] and OSTWALD (**21**) to be for animals, and as OSTERHOUT (**17**) and MAGOWAN (**14**) have found it to be for plants. LOEB found, for instance, that the formation of embryos in the eggs of Fundulus was rendered impossible in a 0.625 *m* solution of NaCl, whereas in a 0.7 *m* solution of the same salt *B. subtilis* makes a fairly good growth and forms a considerable amount of ammonia. As an extreme example of the toxicity of NaCl, OSTERHOUT (**18**) found that zoospores of *Vaucheria sessilis* placed in a 0.0937 *m* solution of NaCl usually died within a few min-

[1] See also **5** and literature there cited.

utes, and further, that even at a dilution of 0.0001 *m* NaCl proved poisonous to the young plants. The results above given serve, however, to confirm again the general principle, formulated by LOEB, of the toxicity of NaCl alone for living organisms.

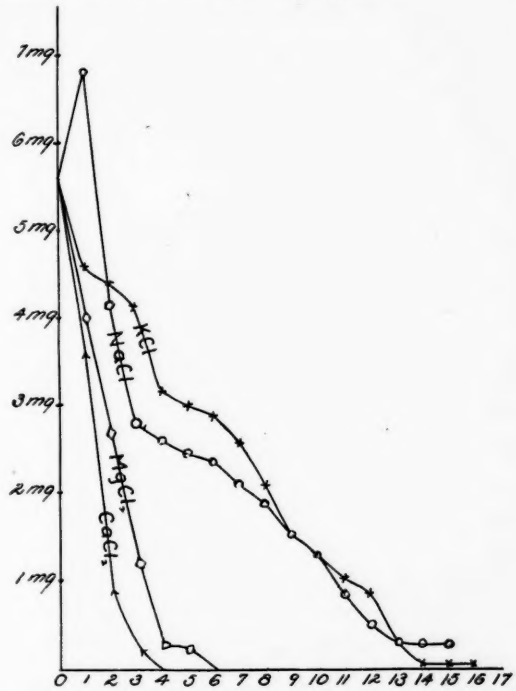

FIG. 1.—Toxicity curves of KCl, NaCl, $MgCl_2$, and $CaCl_2$. The ordinates represent milligrams of ammonia nitrogen. The ordinate at 0 represents the amount of ammonia nitrogen formed in a blank culture (1 per cent. peptone in distilled water). The abscissae represent concentrations in tenths molecular.

SERIES II. CALCIUM CHLORID

Here the experiment was carried out in the same general way as in series I, $CaCl_2$ being substituted for NaCl, and, owing to the extreme toxicity of the former, solutions ranging from 0.1 *m* to 0.6 *m* only were prepared. The ammonia was determined as above and the results are shown in table II.

Here again (see *fig. 1*) we find agreement between the toxic action of $CaCl_2$ on bacteria with that of the same salt and other calcium salts on animals as shown by LOEB (**8**, p. 425) in the case of Fundulus, and by OSTWALD (**21**) in his work on the freshwater Gammarus. It is well to note here also that an examination of the four curves (*fig. 1*) for the salts employed reveals the fact that $CaCl_2$ is easily the most toxic of all for *B. subtilis*. This fact is of especial interest because of its wide disagreement with the facts obtained by experiments on plants with $CaCl_2$, in which MAGOWAN (**14**), for example, found it to be the least toxic of the four salts for wheat (variety Early Genesee). In this respect therefore, if *B. subtilis* may be considered representative, bacteria exhibit the physiological characteristics more typical of animals than of plants, with which they are now classed. We see above, that even at a concentration of 0.3 *m* $CaCl_2$ the formation of ammonia by *B. subtilis* is inhibited.

TABLE II

Numbers represent tenths *m* $CaCl_2$ solution	Milligrams of N formed as NH_3
0	5.60
1	3.15
2	0.91
3	0.21
4	0.00
5	0.00
6	0.00

SERIES III. POTASSIUM CHLORID

The experiment was arranged as those preceding it and the results are shown in table III.

On examining the curve (*fig. 1*) obtained from these results we see at once the strong resemblance of it to that obtained with the solution of NaCl, and although at the concentrations employed KCl exhibits no stimulating effect, it may show it at some concentration lower than 0.1 *m*. This general agreement of the chlorids of K and Na has been found to be even more striking by MAGOWAN (**14**) in a series of experiments on wheat. Here, therefore, *B. subtilis* exhibits physiological characteristics akin to those of the higher plants and differing widely from those of animals, as shown, for

TABLE III

Numbers represent tenths *m* KCl solution	Milligrams of N formed as NH_3
0	5.60
1	4.55
2	4.41
3	4.13
4	3.15
5	3.01
6	2.87
7	2.59
8	2.10
9	1.54
10	1.33
11	0.84
12	0.49
13	0.28
14	0.07
15	0.07
16	0.07

example, by the work of OSTWALD (**21**) in which KCl showed the most extreme toxicity of all the salts tested.

It is also worthy of mention here, as shown above, that the KCl curve declines more gradually than the sodium curve beyond the concentrations of 0.1 *m*.

SERIES IV. MAGNESIUM CHLORID

In the curve drawn on the basis of table IV (*fig. 1*) we notice a parallel to the toxicity curve for $CaCl_2$, except that $MgCl_2$ is not nearly as toxic for *B. subtilis* as the former. While a 0.3 *m* solution of $CaCl_2$ totally inhibits the ammonifying activity of *B. subtilis*, the latter will make a fair growth in a solution of $MgCl_2$ of like concentration and form an appreciable amount of ammonia. Here again, we find agreement between the behavior of *B. subtilis* and Fundulus in solutions of $MgCl_2$, as LOEB (**7**, p. 411) found that in an *m*/2 solution of $MgCl_2$ no embryo develops in the eggs of Fundulus, but that the same degree of toxicity is reached in an *m*/8 solution of $Ca(NO_3)_2$, thus showing the magnesium salt to be less toxic than the calcium salt. On the other hand, as shown by MAGOWAN (**14**), $MgCl_2$ is far the most toxic of the four chlorids employed in experiments on wheat; and in this respect the behavior of *B. subtilis* resembles that of Fundulus rather than that of the higher plants.

TABLE IV

Numbers represent tenths *m* $MgCl_2$ solution	Milligrams of N formed as NH_3
0	5.60
1	4.00
2	2.70
3	1.20
4	0.28
5	0.28
6	0.00

Experiments with binary solutions

The fact that the results obtained were in such striking general agreement with those of the investigators above mentioned on animals and higher plants was sufficient stimulus for further inquiry into the biochemistry of ammonification. It was deemed of interest, therefore, to see if antagonism between salts holds as well for bacteria as it does for the higher forms of life, with the end in view of ascertaining whether balanced solutions are necessary for bacteria, and whether solutions balanced for the other forms of life investigated will prove the same for bacteria. The remainder of this paper will deal with the antagonistic effects of one salt on another in binary solutions, while

the establishment of a balanced solution, with other work now contemplated by the writer, will form the subject of another article.

SERIES V. POTASSIUM CHLORID *vs.* CALCIUM CHLORID

In these experiments the technique was somewhat different from the foregoing. All the salts were used at a concentration of 0.35 m[2] and mixed with each other in various proportions in Erlenmeyer flasks of 250^{cc} capacity. Two or three liters each of KCl and $CaCl_2$ solution of the concentration above noted were made up to contain 0.75 per cent. peptone.[3] In each flask of the first half of the series were placed 100^{cc} of the KCl-peptone solution. To no. 1 nothing was added; to the others up to no. 6 there were added respectively 5, 10, 25, 50, and 100^{cc} of the $CaCl_2$-peptone solution. Then beginning at the other end of the series each flask received 100^{cc} of the $CaCl_2$-peptone solution. Flask no. 11 contained this alone; to the preceding ones in order were added respectively 5, 10, 25, 50, and 100^{cc} KCl-peptone solution, the two halves of the series meeting in no. 6, as shown in the curve (*fig. 2*) and table V, at the combination of 100^{cc} of each solution. In order to keep the volume the same in all cultures they were thoroughly mixed, and enough solution drawn off with the pipette, where additions were made, to make all the culture

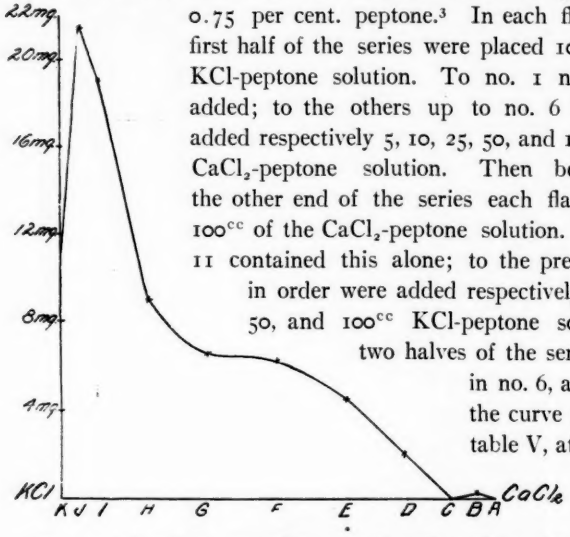

FIG. 2.—Antagonism curve, KCl *vs.* $CaCl_2$. The ordinate at K represents the ammonia nitrogen in milligrams formed in a pure KCl solution. The ordinate at A represents the amount of ammonia nitrogen formed in a pure $CaCl_2$ solution, and the ordinates at the intermediate points represent the amounts formed in various combinations of the two salts as indicated by the corresponding letters in table V.

[2] This concentration was chosen because it was about the concentration of NaCl in sea water of San Francisco Bay.

[3] This concentration of peptone was used in all the experiments with binary solutions.

solutions have a uniform bulk of 100^{cc}, thus avoiding any differences in the supply of oxygen to the bacteria by having an equal surface of liquid exposed to the air in each flask.

As in the case of the single salt solutions, duplicates were run on all the cultures, and also sterile controls, so as to allow of a determination of the ammonia actually formed by the bacteria. The solutions were all sterilized in the autoclave at 1.25 atmospheres, inoculated as above, and incubated for two and a half days at 28° to 29° C., after which they were distilled, as in the case of the single salt cultures, and the ammonia determined. The results obtained follow, with the curve plotted from them in accordance with the arrange-

TABLE V

ALL QUANTITIES GIVEN REFER TO CUBIC CENTIMETERS OF 0.35 *m* SOLUTIONS

Culture solution	Corresponding points on curve	Milligrams of N formed as NH_3
100 KCl	K	10.92
100 KCl + 5 $CaCl_2$	J	21.46
100 KCl + 10 $CaCl_2$	I	18.83
100 KCl + 25 $CaCl_2$	H	9.00
100 KCl + 50 $CaCl_2$	G	6.44
100 KCl + 100 $CaCl_2$	F	6.30
50 KCl + 100 $CaCl_2$	E	4.55
25 KCl + 100 $CaCl_2$	D	0.25
10 KCl + 100 $CaCl_2$	C	0.14
5 KCl + 100 $CaCl_2$	B	0.25
100 $CaCl_2$	A	0.00

ment employed by OSTERHOUT (**19**). The letters along the axis of abscissas represent a given combination of the two salts as indicated in the table, and the ammonia formed is laid off on the axis of ordinates in numbers representing milligrams.

We see at a glance (*fig. 2*) that there is a strong antagonism

between $CaCl_2$ and KCl. OSTERHOUT (**20**) obtained a similar curve for the antagonism between the same two salts in his experiments on wheat. It is particularly interesting to note that both for wheat and for bacteria the maximum point of the curve is at the combination of 100^{cc} KCl solution and 5^{cc} $CaCl_2$ solution, notwithstanding the wide difference between the materials employed in the two cases, and further despite the fact that the Ca ions are most toxic for *B. subtilis* and least toxic for the wheat.

Again we find the strong antagonism above obtained is in striking accord with the results of LOEB in similar experiments on animals, both as to development (**7**) and as to muscular contraction (**6**).

SERIES VI. SODIUM CHLORID *vs.* MAGNESIUM CHLORID

In this series the experiment was arranged and carried out in the same manner as the one preceding, except that the salts used were different. The ammonia formed at the end of the period of incubation was determined with the following results:

TABLE VI

ALL QUANTITIES GIVEN REFER TO CUBIC CENTIMETERS OF 0.35 *m* SOLUTIONS

Culture solution	Corresponding points on curve	Milligrams of N formed as NH_3
100 NaCl	K	22.36
100 NaCl + 5 $MgCl_2$	J	23.75
100 NaCl + 10 $MgCl_2$	I	28.67
100 NaCl + 25 $MgCl_2$	H	24.56
100 NaCl + 50 $MgCl_2$	G	18.67
100 NaCl + 100 $MgCl_2$	F	10.49
50 NaCl + 100 $MgCl_2$	E	4.84
25 NaCl + 100 $MgCl_2$	D	3.80
10 NaCl + 100 $MgCl_2$	C	3.54
5 NaCl + 100 $MgCl_2$	B	3.36
100 $MgCl_2$	A	2.13

In the curve plotted from table VI (*fig. 3*), we note again a strong antagonism between the two salts tested, though it is not as marked as in the last series. The curve proves to be more regular than the last, probably owing to the fact that there was practically no variation in the temperature throughout the period of incubation. As LIPMAN (4) has demonstrated, a constant temperature and equal periods of incubation are essential factors in quantitative work in ammonification, if results are to be considered comparable.

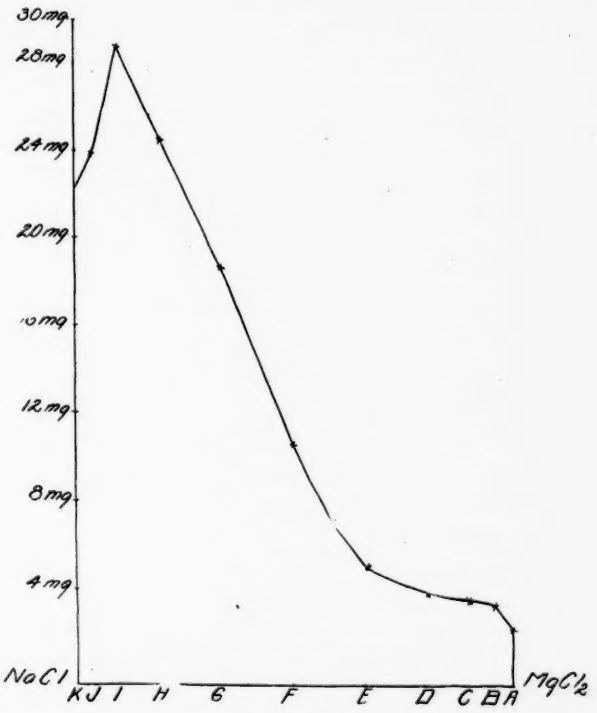

FIG. 3.—Antagonism curve, NaCl *vs.* $MgCl_2$. The ordinate at K represents the ammonia nitrogen in milligrams formed in a pure NaCl solution. The ordinate at A represents the amount of ammonia nitrogen formed in a pure $MgCl_2$ solution, and the ordinates at the intermediate points represent the amounts formed in various combinations of the two salts as indicated by the corresponding letters in table VI.

It is a significant fact that here again the maximum point on the curve nearly coincides with that of a similar curve obtained by OSTERHOUT (**19**) in his experiments with the same salts on root development in wheat, and exactly coincides in the case of a fungus (*Botrytis cinerea*); and though OSTERHOUT employed such widely varying concentrations as 0.12 *m* in the case of the wheat and 1.5 *m* in Botrytis, the maximum development was reached in a mixture of 10^{cc} of the $MgCl_2$ solution (or 7.5^{cc} for wheat) and 100^{cc} of the NaCl solution, just as was the case in ammonification by *B. subtilis*.

In his experiments on the eggs of Fundulus and the sea-urchin (Arbacia), LOEB (**10**) found that in a mixture of 98^{cc} $5n/8$ NaCl and 2^{cc} $10n/8$ $MgCl_2$ all the eggs of Fundulus form embryos, whereas in pure NaCl or $MgCl_2$ solutions alone no embryos would form, and even in a mixture of equal parts of the above-mentioned solutions 75 per cent. of the eggs formed embryos. On the other hand, OSTWALD (**21**) found in his work on the freshwater Gammarus that, so far from exercising an antagonistic effect on each other, the combination of Mg and Na chlorids proved more poisonous than either alone.

SERIES VII. MAGNESIUM CHLORID *vs.* CALCIUM CHLORID

The arrangement of the experiment and the ammonia determinations were carried out in a manner similar to that employed in the two preceding series, two bivalent salts being tested this time. The results were as shown in table VII, p. 117.

By an examination of the curve drawn on the basis of table VII (*fig. 4*) we are confronted by the very striking instance of lack of antagonism between the two salts. On the contrary, there is a constant increase of the toxic properties of each when the other is added to it in increasing amounts. In this exceptional behavior, so far as the writer can ascertain, *B. subtilis* (and probably all the ammonifiers) stand alone, when their physiological efficiency in such salt mixtures is compared with that of the higher plants and animals. No instance of such behavior on the part of any member of the latter two groups of organisms has come to my notice in reviewing the results of similar researches.

An antagonism between $CaCl_2$ and $MgCl_2$, though slight, was

found to be none the less definite by LOEB (**6**) in experiments which showed that sea-urchin blastulae and gastrulae would swim about

TABLE VII

ALL QUANTITIES GIVEN REFER TO CUBIC CENTIMETERS OF 0.35 *m* SOLUTIONS

Culture solution	Corresponding points on curve	Milligrams of N formed as NH_3
100 $MgCl_2$	K	3.08
100 $MgCl_2$ } 5 $CaCl_2$ }	J	2.59
100 $MgCl_2$ } 10 $CaCl_2$ }	I	1.68
100 $MgCl_2$ } 25 $CaCl_2$ }	H	.98
100 $MgCl_2$ } 50 $CaCl_2$ }	G	.21
100 $MgCl_2$ } 100 $CaCl_2$ }	F	.07
50 $MgCl_2$ } 100 $CaCl_2$ }	E	.00
25 $MgCl_2$ } 100 $CaCl_2$ }	D	.00
10 $MgCl_2$ } 100 $CaCl_2$ }	C	.00
5 $MgCl_2$ } 100 $CaCl_2$ }	B	.00
100 $CaCl_2$	A	.49

in a mixture of the salts above mentioned for forty-eight hours, while each salt by itself would immediately prove poisonous at the concen-

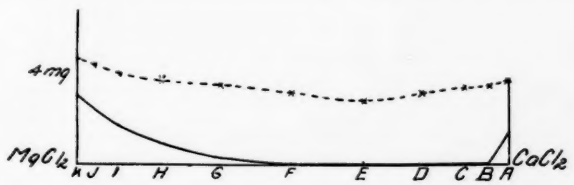

FIG. 4.—Non-antagonism curves, $CaCl_2$ *vs.* $MgCl_2$. The ordinates at K represent the ammonia nitrogen in milligrams formed in a pure $MgCl_2$ solution. The ordinate at A represents the amount of ammonia nitrogen formed in a pure $CaCl_2$ solution, and the ordinates at the intermediate points represent the amounts formed in various combinations of the two salts as indicated by the corresponding letters in tables VII (unbroken line) and VIII (dotted line).

tration employed in the combination. Another interesting case in point may be noted in the experiments of the same investigator on Polyorchis (**11**), a jelly-fish of San Francisco Bay. In a solution of 50^{cc} NaCl + 6^{cc} $MgCl_2$ + 1^{cc} $CaCl_2$, the rhythmical contractions of the margin go on normally, but with a slight increase of $CaCl_2$ the contractions are inhibited, and when 5^{cc} of a $3m/8$ solution of $CaCl_2$ are added, they are completely suppressed. On the other hand, when the margin of the fish, containing the sense organs and the central nervous system, is cut off, $CaCl_2$ exercises a stimulating action on the isolated center of the fish, and contractions will go on normally; but when $MgCl_2$ is added to the solution, in the ratio of 4 parts $MgCl_2$ to 1 part $CaCl_2$, the stimulating action of the $CaCl_2$ is suppressed and contractions cease. In both cases, therefore, there is evidence of a definite antagonism between Ca and Mg. Likewise, LILLIE (**3**) proved the existence of antagonism between the two salts, when he found that the ciliary activity of the larvae of Arenicola would go on normally for some time in a mixture of approximately 4 parts $MgCl_2$ to 1 part $CaCl_2$, whereas it would immediately cease if either of the salts at the same concentration was present alone.

Again, we find the well-known researches of LOEW and his pupils (**12, 13**), and later the researches of KEARNEY and CAMERON (**1**), which show in the higher plants the strong antagonism between calcium and magnesium. The last-named investigators found, in their experiments with the white lupin (*Lupinus albus*) and with alfalfa (*Medicago sativa*), that when $CaCl_2$ was added to $MgSO_4$ in about equal proportions, the plants exhibited about 160 times the tolerance for the latter salt that they did in solutions of $MgSO_4$ alone. They found, further, that the antagonism between $CaCl_2$ and $MgCl_2$, though not so great (increasing the tolerance about 40 times), was nevertheless very marked, and where $CaSO_4$ replaced $CaCl_2$ the antagonism was very much greater between Ca and Mg than in either of the cases above cited.

I wish to cite only one more case, which emphasizes by strong contrast the exceptional results obtained above in experiments with *B. subtilis;* that is, the results of highly ingenious experiments on rabbits and a monkey by MELTZER and AUER (**16**) showing the antagonistic effect of calcium on the inhibitory effect of magnesium. As a

typical instance may be cited experiment 1 of their series, in which about 13^{cc} of an $m/1$ solution of $MgCl_2$ was injected subcutaneously. Less than half an hour later there was produced general anaesthesia, with all the attending symptoms.. When 2^{cc} of a solution of $m/8$ $CaCl_2$ was injected into the ear vein the rabbit was again breathing normally, and when 8^{cc} had been given the animal sat up and appeared entirely recovered, except for a stiffness in the hind legs.

In these experiments, some of which were even more striking than the one cited, MELTZER and AUER employed, besides the chlorids of Ca and Mg, the acetate and nitrate of the former, and the acetate, nitrate, and sulfate of the latter. The same strong antagonism was noted in all cases.

TABLE VIII

ALL QUANTITIES GIVEN REFER TO CUBIC CENTIMETERS OF 0.35 m SOLUTIONS

Culture solution	Corresponding points on curve	Milligrams of N formed as NH_3
100 $MgCl_2$	K	4.76
100 $MgCl_2$ + 5 $CaCl_2$	J	4.48
100 $MgCl_2$ + 10 $CaCl_2$	I	4.20
100 $MgCl_2$ + 25 $CaCl_2$	H	3.78
100 $MgCl_2$ + 50 $CaCl_2$	G	3.64
100 $MgCl_2$ + 100 $CaCl_2$	F	3.22
50 $MgCl_2$ + 100 $CaCl_2$	E	3.08
25 $MgCl_2$ + 100 $CaCl_2$	D	3.29
10 $MgCl_2$ + 100 $CaCl_2$	C	3.43
5 $MgCl_2$ + 100 $CaCl_2$	B	3.57
100 $CaCl_2$	A	3.78

In addition to the confirmation of the results stated above in experiments with the same material, one series was also carried out with a culture of *B. subtilis* obtained from New Jersey, and with salt solutions made up from a different grade of chemically pure salt.

As can be seen from the following table and also from *fig. 4*, the results fully confirm those above given; and though the absolute amounts are different, the results are relatively the same.

It may be of interest to note here that *B. subtilis* from a 24-hour peptone agar slope was examined in hanging drops of molecular solutions of magnesium chlorid and calcium chlorid, and the organisms showed no perceptible ill effects from the action of the solution. The ciliary movements appeared normal even after 24 hours in the hanging drop. It was noticed, however, that there was little or no division during the 24 hours and it is likely that the calcium and magnesium salts exercise their toxic effects, partly at least, by inhibiting reproduction, since the ciliary movements seemed to go on without interruption. These remarks, however, are based on too meager experimental evidence to be anything else than conjecture at present, but they serve to indicate a field of most interesting research.

Though they are not analogous to the lack of antagonism between Ca and Mg shown above, it is interesting to note two cases on record, in which the addition of one salt to another made a combination more toxic than either. One case is that cited above from OSTWALD'S experiments on the freshwater Gammarus, in which a combination of $MgCl_2$ and NaCl in solution was more toxic to that animal than either of these in solution alone. The other case is that noted in the experiments of KRÖNIG and PAUL (**2**), who found that the value of mercuric sulfate, acetate, and nitrate as disinfectants was enhanced by the addition of small amounts of the chlorids of the alkalies (K and Na); but, on the other hand, that the addition of the same chlorids to $HgCl_2$ reduced considerably the disinfecting powers of the latter.

The first instance is not analogous to the results of the writer, because one of the salts used was different, and the experiment was carried out under conditions so totally different that the value of a comparison is doubtful. In the second instance, as KRÖNIG and PAUL themselves suggest in the same article, the increase of toxicity is not necessarily owing to a lack of antagonism between the two salts, but rather to the formation of complex double salts of mercury, which are characteristic of that element, and therefore this again cannot be compared with the lack of antagonism between Ca and Mg above noted.

SERIES VIII. SODIUM CHLORID *vs.* POTASSIUM CHLORID

This series was carried out to determine the antagonistic action between KCl and NaCl and was arranged in the same manner as the foregoing series. The ammonia determinations gave the following results:

TABLE IX

ALL QUANTITIES GIVEN REFER TO CUBIC CENTIMETERS OF 0.35 *m* SOLUTIONS

Culture solution	Corresponding points on curve	Milligrams of N formed as NH_3
100 NaCl	K	10.99
100 NaCl + 5 KCl	J	11.62
100 NaCl + 10 KCl	I	10.50
100 NaCl + 25 KCl	H	10.08
100 NaCl + 50 KCl	G	9.66
100 NaCl + 100 KCl	F	11.62
50 NaCl + 100 KCl	E	17.57
25 NaCl + 100 KCl	D	14.42
10 NaCl + 100 KCl	C	13.44
5 NaCl + 100 KCl	B	11.41
100 KCl	A	10.57

That the like valences of the two salts employed do not prevent antagonistic action in the case of *B. subtilis* (as may have been surmised from results in the last series) can be seen from the curve in *fig. 5*. Here again, there is a marked resemblance between the effect of this combination of metallic salts on *B. subtilis* and on animals and higher plants. LILLIE, for example, showed (**3**) that the ciliary activity of the larvae of Arenicola, which was inhibited in solutions of either KCl or NaCl alone, went on normally when solutions of the two at the same concentrations were mixed in the proportion of 20 parts NaCl to 8 parts KCl.

LOEB (**9**) and OSTWALD (**22**) also found, in working on a marine

and a freshwater Gammarus respectively, that a distinct antagonism exists between NaCl and KCl. Among plants we find that the work of OSTERHOUT (**20**) also shows some antagonism between KCl and NaCl, and here again it is significant to note that there are two maxima.

The curve in *fig. 5* shows an unusually gradual rise and decline.

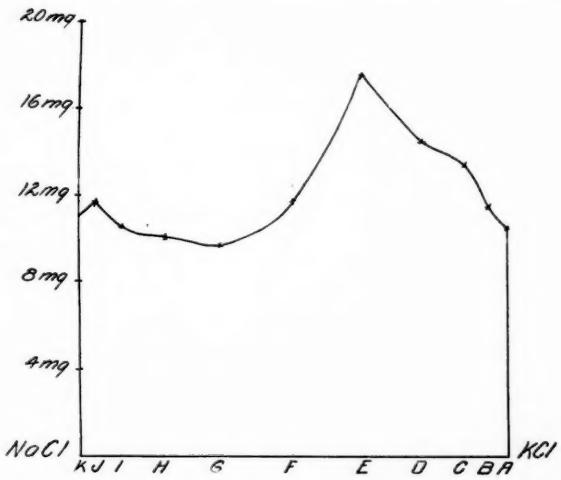

FIG. 5.—Antagonism curve, NaCl *vs.* KCl. The ordinates at K represent the ammonia nitrogen in milligrams formed in a pure NaCl solution. The ordinate at A represents the amount of ammonia nitrogen formed in a pure KCl solution, and the ordinates at the intermediate points represent the amounts formed in various combinations of the two salts as indicated by the corresponding letters in table IX.

This is characteristic of the Na and K chlorids used singly, where they become gradually more and more toxic with the increase of concentration. It is consequently in strong contrast to the sharp decline of the calcium and magnesium curves, especially that of the former. In this curve, as above noted, there are two maximal points, one at the combination of 100^{cc} of NaCl + 5^{cc} KCl, and another at the combination of 100^{cc} KCl + 50^{cc} NaCl. In three confirmatory series on this experiment, the maximal points were in each case obtained at the same combinations.

Discussion

Briefly reviewing the results with *B. subtilis* above given, we note their significance from the scientific as well as the practical standpoint. Of the four chlorids tested singly, NaCl is the only one stimulating ammonification at the concentrations employed. It is not unlikely, however, that KCl has a similar effect at a slightly lower concentration.

$CaCl_2$ is the most toxic of the chlorids used. In this feature, *B. subtilis* appears to resemble animals, for which calcium is very toxic, and not plants; since for plants, with which bacteria are now classed, calcium is the least toxic of the four chlorids. This fact may have a bearing on the future classification of bacteria.

The strong antagonism exhibited in some of the combinations of salts employed speaks eloquently for the fact that balanced solutions are as necessary for the optimal development of bacteria and allied forms as for the higher plants and animals, a fact which has been denied in a recent publication of LOEW and ASO (**13**). This is of the greatest practical significance when applied to soil bacteria, and especially those of alkali soils, in which, owing to the excessive amount of one or more salts, the bacterial activity is inhibited, and consequently plant food is not made available to the higher plants.

Because the salts experimented with are all found in our soils in larger or smaller amounts, and in some soils are present in excess, it is a matter of practical importance to apply the results of researches on the physiology of plants and bacteria to improve such alkali lands. There is no doubt in my mind that when we have learned to coordinate the results of researches on plants and soil bacteria, and to apply them in the field, we shall have at our command a method for the control and profitable cultivation of alkali lands, of which so many thousands of acres are merely vast wastes at the present time.

Lastly, the results of these experiments are significant because they open up an unexplored field of bacterial physiology, in which further researches will teach us much.

Summary

1. Each of the four chlorids ($CaCl_2$, $MgCl_2$, KCl, NaCl) is toxic for *B. subtilis*, in the order given, the first being the most toxic and

the fourth the least. This is quite different from the results with higher plants, where magnesium is the most toxic and calcium the least.

2. A marked antagonism exists between Ca and K, Mg and Na, K and Na.

3. No antagonism exists between Mg and Ca, but the toxic effect of each is increased by addition of the other to it. This is just the opposite of what has hitherto been found for plants.

In conclusion, the writer desires to express his indebtedness to Prof. W. J. V. OSTERHOUT, at whose instance these researches were begun, for helpful suggestions and kindly criticism throughout the course of the investigations.

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THE GAMETOPHYTES OF CALOPOGON

LULA PACE

(WITH PLATES VII–IX)

Photolith plates 2nd edition

This paper reports a continuation of the work on certain orchids begun in 1906. The peculiarities in the development of the megaspores, and in the number and origin of the nuclei in the embryo sac found in Cypripedium (**14**) made it seem desirable to continue the investigation.

The same methods were used as in the work on Cypripedium. The material of *Calopogon pulchellus* R. Br. was collected near Chicago in the summer of 1906. The usual chromacetic and alcohol-formalin solutions were carried to the field and the material was killed as collected. The greater part of it has been cut in serial sections five to seven microns thick, and stained in safranin and gentian violet.

MEGASPORES

The ovules are very numerous and even smaller than those of Cypripedium, the mother cell being five to ten microns in diameter. The archesporium does not seem to be differentiated early. *Fig. 1* shows an ovule pretty well advanced, and yet it is not possible to distinguish an archesporial cell by any difference in staining. The one drawn shows the greatest difference in size in favor of the usual archesporial cell, but other ovules in the same ovary show the other cells of the axial row to be larger; so here also it is probably the cell that is approaching mitosis that is larger. *Fig. 2* is taken from another ovary in which the ovules are apparently in the same stage as in *fig. 1*. This one suggests a more advanced stage by the broadening of the ovule. Yet this may be related to the appearance of more than the one mother cell, for these ovules with two sporogenous cells are broader, as will be seen later. The first undoubted archesporium seen was in the stage shown in *fig. 3*. Here the ovule is much larger than in the first two figures and the first integument is beginning. In this ovary more than half of the ovules were in this stage, the archesporial cell being large and showing difference in staining reaction.

The other ovules were in the mother cell stage (*fig. 4*). The integument is only a trifle more advanced in the latter than in the former, but the nuclei are in all stages of synapsis. The archesporial cell may become the mother cell without division, and no parietal cell was ever found. But it is possible that in some cases it divides; for an archesporium of more than one cell was rare, being found only once (*fig. 26*), and yet several ovules with two mother cells in position to have been formed by division of a single archesporial cell were found (*figs. 27, 29*). It is evident that these must have originated by a division of the archesporial cell, or the archesporium must have consisted of two cells.

A more remarkable case still is that in which two distinct sporogenous areas are differentiated in the same ovule (*figs. 28–31*). *Fig. 31* shows several cells of nucellar tissue between the two mother cells, the cut being across the ovule in a different direction from that of *fig. 28*. A somewhat later stage, similar to *fig. 28*, is shown in *fig. 30*. *Fig. 32* shows at least one of the mother cells divided, and one of the resulting daughter cells increasing for the second division, the other disintegrating in the usual fashion. The presence of more than one mother cell is not rare in my material. Of about sixty ovaries cut, thirteen showed ovules with two mother cells, or stages derived from this condition. In these thirteen ovaries, the ovules showing this condition vary from one to seven, giving a total of thirty-seven. Of these thirty-seven, twenty-one are similar to *figs. 28* and *31*, and sixteen resemble *figs. 27* and *29*. There was in all probability double this number, for only those were counted in which both cells appeared in the same section. No attempt was made to trace the ovules from section to section for this condition, and it is evident that there are more chances against getting both in the same section than in favor of it. *Fig. 31* is a drawing from a single section, but the whole ovule was traced carefully from section to section for evidence of the coalescence of two ovules. The only abnormal appearance was the unusually broad funiculus. *Fig. 33* shows the megaspores derived from the two adjacent mother cells. In each case the chalazal megaspore is developing and the other three are disintegrating. In the lower or micropylar group the megaspores are in a row—the usual row of four, except that the wall is lacking between the two upper megaspore

nuclei. In the upper or chalazal group two of them lie side by side without a separating wall. The upper two are in the normal position, but the usual separating wall is lacking. *Fig. 34* seems to show an embryo sac of two nuclei derived from the chalazal group of two mother cells. The three disintegrating nuclei are probably megaspore nuclei, and the micropylar mother cell has not divided. *Fig. 35* is taken from a much younger ovule, but a very broad one. It seems to be the product of two sporogenous cells, the chalazal mother cell having divided to form the two daughter nuclei, the micropylar mother cell being still in metaphase. It is possible that this is the result of the second division of the chalazal daughter cell without walls separating the two megaspore nuclei, the micropylar daughter cell being still in mitosis. But the chromosomes in this nucleus give every appearance of the heterotypic division, and the ovule is so unusual in appearance that it seems to have had two mother cells.

Synapsis continues for some time, if the extent of development of the integument be considered proof (*figs. 4, 5*). The division of the mother cell takes place in the usual way (*figs. 5–10*), giving the two daughter cells (*fig. 11*). The number of chromosomes is apparently thirteen (*fig. 6*), although only a few counts were made, but this one seemed unusually distinct, so it is probably the correct number. Several counts were attempted in a sporophytic area, giving approximately twenty-six. It is probable that sometimes the wall separating the daughter nuclei fails to appear. *Fig. 9* would be expected to show some evidence of wall formation, if it were going to appear. *Fig. 15* shows the division completed, with no suggestion of a wall. *Fig. 16* gives no wall. Several examples of this failure of the wall were found, and in others it seemed very faint and probably disappeared.

In many plants the chalazal daughter cell divides; but in Calopogon it seems quite common for both to divide, although the micropylar cell is even then somewhat smaller than the chalazal cell (*figs. 13, 14*). These divisions may occur at the same time as in the above figures, or either may precede the other. *Figs. 17* and *18* show the chalazal cell dividing first, the former showing the micropylar cell somewhat more advanced than the latter. In *fig. 19* the micropylar daughter cell has almost completed the division, while the chalazal

cell is still in the spirem stage. This does not appear so often as the other in my material. At this division of the daughter cells the walls begin to form in the usual way (*figs. 14, 18, 19*), though in a few instances no evidence of wall formation was seen (*figs. 17, 20*). But the walls all disappear, if they were ever found in these cells (*figs. 20, 28*), for no case of a wall at this stage was seen, though hundreds of examples like those figured were found. After this division the micropylar nucleus from the inner daughter cell begins to disintegrate (*figs. 21, 22*), although it may sometimes divide (*fig. 24*). *Fig. 23* shows both daughter cells divided, with one megaspore nucleus in each case disintegrating. While apparently two of the megaspores are active, one of them is already in advance of the other, judging by size of nucleus and cell. *Fig. 24* shows the micropylar daughter cell with the nucleus in metaphase, while *fig. 25* is only late spirem. Probably the former would have completed the division. *Figs. 24* and *25* are also interesting as the only cases seen in which the megaspore nucleus that usually disintegrates in the sac shows evidence of further development. In the first it has divided, while in the second it is in the spirem stage, yet in both cases it is evidently disintegrating. The megaspore that is to form the sac in *fig. 25* is in mitosis with the spindle well formed.

EMBRYO SAC

As the walls disappear, or never develop, in the division of the daughter cells, the two megaspore nuclei are left in the embryo sac. The micropylar nucleus apparently always disintegrates, as does the cytoplasm about this nucleus (*figs. 21, 22, 23, 48*). This leaves only one megaspore nucleus, probably one might say only one megaspore, to enter into the organization of the embryo sac. This nucleus divides, giving a sac with two nuclei (*fig. 46*). The three other bodies in the sac are probably the three disintegrating megaspore nuclei. The two nuclei of the sac divide simultaneously (*fig. 47*), giving a four-nucleate sac (*fig. 48*). This figure is interesting because the three disintegrating megaspore nuclei are easily identified at this late stage. *Fig. 49* shows about the same stage, except that the sac has increased in size and the nuclei are preparing for the next division, although there are still traces of the spindles of the preceding

mitosis. These spindles show no traces of wall formation. The micropylar daughter cell did not divide here, although the nucleus completed the prophase, even forming the chromosomes. There is just a trace of the disintegrating megaspore nucleus in the sac. The four nuclei in the sac increase in size and divide in the usual fashion (*fig. 50*). The two dark bodies in the lower part of this sac are probably the remains of the micropylar daughter cell and the disintegrating megaspore nucleus. This division gives eight nuclei in the sac (*fig. 51*). By comparing *figs. 51* and *52* it can be seen that one of the four nuclei at the lower end passes up toward the center, and the sister to the egg moves down in the usual position for the two polar nuclei. The eight nuclei arrange themselves in the usual way—an egg apparatus of two synergids and the egg in the micropylar end of the sac, three antipodals in the chalazal end, and the two polars near the center (*figs. 52, 53*). *Fig. 53* shows a pollen tube already forcing its way between the integuments, about halfway to the sac.

MICROSPORES AND MALE GAMETOPHYTE

The pollen in Calopogon is in masses or pollinia, as it is in many of the orchids. Each massula is apparently the group of cells resulting from the division of each sporogenous cell (*fig. 36*). The whole sporogenous area probably reaches the mother cell stage at the same time (*fig. 37*). The mother cells divide in the usual way, by the so-called simultaneous division, which is thought to be more characteristic of dicotyledons than of monocotyledons, although found in both groups (COULTER and CHAMBERLAIN **7**, p. 121). This gives the tetrad form in some cases, but in others the four spores lie in any plane (*figs. 38–42*). The microspore nucleus divides into the tube and generative nuclei while the pollen is still in the sac (*fig. 43*). Pollen tubes are numerous in the ovary, in some cases at least, when ovules are still in the mother cell stage, like *fig. 5*, although no pollen tube was ever seen in contact with an ovule at this stage. *Figs. 41* and *42* are examples of pollen tubes within the ovary, before entering the ovules. In these the generative nucleus had already divided into the two male nuclei, so it is probable that this division takes place when the pollen grain germinates.

FERTILIZATION

As has been said, the embryo sac when ready for fertilization contains eight nuclei, arranged in the usual fashion—the egg apparatus of two synergids and an egg in the micropylar end, three antipodals at the opposite end, and the two polars about the center of the sac (*fig. 53*). When the pollen tube is entering the sac, the egg and the polar nuclei may be in late spirem stage (*fig. 54*). In *fig. 55* the two polars have already formed chromosomes. *Fig. 56* shows the fusion of a male nucleus with the egg, and the triple fusion in the center of the sac is undoubtedly that of the two polars and the second male nucleus. Not very much material was examined at this stage. But in all that was seen there was every indication that this is the usual condition.

DISCUSSION

Sporogenous cells.—BOWER (**3**) states that a multicellular archesporium is found in several of the archichlamydeous dicotyledons, especially in Amentiferae, Rananculaceae, Rosaceae; but that it is apparently rare in more advanced dicotyledons and in the monocotyledons. GUIGNARD (**9**) reports *Ornithogalum pyrenaicum* with an archesporium of two cells, only one of which gets beyond the archesporial stage. BARNARD (**1**) reports two embryo sacs in *Lilium candidum*. COULTER and CHAMBERLAIN (**7**, p. 61) say that they have seen one preparation of *Lilium philadelphicum* with three archesporial cells and another with five; but no figures are given. FERGUSON (**8**) in a note figures two mother cells in *Lilium longiflorum* with intervening nucellar tissue. But, as has already been shown, there are many such cases in Calopogon, thirty-seven cases of two mother cells or stages evidently derived from them being seen, in thirteen ovaries out of about sixty cut, and this probably represents less than half the number actually present. So while this condition is very far from being the usual one in my material, it could hardly be called rare, and therefore abnormal. It seems best to regard it as a primitive character that has been retained, or at least not entirely eliminated. SARGANT (**15**) and others hold that monocotyledons are derived from dicotyledons. This occasional appearance of a multicellular archesporium may indicate that the dicotyledons from which these came

still had a multicellular archesporium, as the more primitive dicotyledons do yet.

Megaspores.—There are usually four megaspore nuclei formed. However, in a few instances, only one daughter cell divided. In many cases the wall separating these megaspore nuclei at the second division was seen to be forming (*figs. 14, 16, 19*) but always it had disappeared at maturity (*figs. 20, 23*). In a few cases there was no indication of a wall forming (*figs. 15, 17, 20*). This omission of all cell formation in the second division is the condition found in Cypripedium (**14**). The fact that Calopogon usually has the four megaspore nuclei was rather unexpected in this highly specialized group of the monocotyledons. For the tendency is not only to a row of three, due to the failure of one of the daughter nuclei to divide, but to the condition known as the mother cell functioning as a megaspore (COULTER and CHAMBERLAIN **7,** p. 80), which is common in monocotyledons. SCHNIEWIND-THIES (**16**) has shown that in Lilium the heterotypic division takes place within the embryo sac, only ephemeral walls being formed in either of the divisions from the mother cell to the megaspore. In Cypripedium it was found (**14**) that no wall appeared at the second division, and that two megaspore nuclei are used in forming the embryo sac. COULTER has shown (**6**) that this may be a very important point in connection with embryo sacs of more than eight nuclei. For if more than one megaspore nucleus enters into the organization of the sac, it is evident that more than eight nuclei in the sac would be the result of the usual five divisions from mother cell to egg found in angiosperms. So that while Cypripedium with its four-nucleate sac seems more reduced than Lilium, it really has just the same number of divisions from mother cell to egg (**14**).

BROWN (**4**) objects to calling these nuclei in Cypripedium megaspore nuclei, yet he proceeds to do it in Peperomia, although there is only the additional evidence of ephemeral walls. JOHNSON (**11**) has also shown these four nuclei in the tetrad position in Peperomia. The origin of the nuclei is identical in both Cypripedium and Peperomia, with the usual megaspore story except in the matter of wall—a mother cell passing from synapsis through the heterotypic and homotypic divisions. In Cypripedium a permanent wall appears at the heterotypic division, but none at all at the homotypic; in Peperomia

ephemeral walls appear at both divisions. This gives apparently four megaspore nuclei forming the sac in Peperomia, while only two enter into its organization in Cypripedium. It might be better to select another name for these nuclei, but it would always involve the explanation of their similarity to megaspore nuclei, with apparently no gain in clearness. For our notion of spores, extending back through pteridophytes and bryophytes, is that when a mother cell divides there are four spores produced, the spore being the cell formed by the heterotypic and homotypic divisions. The matter of walls would not seem to be so important as the behavior of the nuclear materials; for all sorts of variation in the kinds and shapes of these are found, these differences being related apparently to the environment of the spore. The egg is of course a descendant of only one megaspore, however many may enter into the construction of the sac. And it seems to involve less change from what have seemed to be the essentials to regard these as megaspore nuclei than to conceive of a mother cell functioning directly as a megaspore and yet proceeding to the very same heterotypic and homotypic divisions. This is more evident still when Calopogon is taken into consideration, as it seems to present a stage that might be considered intermediate between Cypripedium and the usual one. For here there are certainly two megaspore nuclei in the sac, one finally disintegrating, although some evidence of its division is present in two cases examined (*figs. 24, 25*).

This lack of walls is not very rare. LLOYD (**12**) reports them as usually absent in Rubiaceae, although the tetrad is formed. Here only one megaspore nucleus functions. The same condition is reported by CANNON (**5**) in Avena, and by SMITH (**17**) in Eichhornia. WIEGAND (**19**) finds the second divisions unaccompanied by walls in *Potamogeton foliosus*, and HOLFERTY (**10**) finds the wall omitted in the division of the micropylar daughter cell in *Potamogeton natans*. BILLINGS (**2**) reports one case of megaspores without separating walls in *Tillandsia usneoides*. And the many cases where the mother cell is said to function as a megaspore seem to represent the same condition, except that the non-functioning megaspores not only do not disintegrate but even divide and thus contribute to the contents of the embryo sac.

In Calopogon only one megaspore nucleus is used in forming

the sac, though two are certainly in it (*figs. 21, 22*), just as in Cypripedium. Both may divide, though no evidence beyond a late spirem or metaphase stage was seen, except in one case (*fig. 24*), and here the usual disintegration was taking place. So in this respect Calopogon might be regarded as suggesting what may have taken place in the ancestry of Cypripedium, as it passed from the usual tetrad or row of three to its present condition.

Fertilization.—So-called double fertilization is present here as in Cypripedium. It will be remembered that NAWASCHIN (**13**) reports it lacking in certain tropical orchids, while STRASBURGER (**18**) found it in orchids he investigated.

SUMMARY

1. There is usually one sporogenous cell which becomes the mother cell. But in many cases two mother cells are found, either contiguous or with nucellar tissue between.

2. Four megaspore nuclei are usually formed; occasionally the micropylar daughter cells in the second division either never appear or are ephemeral. This leaves two megaspore nuclei in the sac. But three of these megaspore nuclei disintegrate, the two from the micropylar daughter cell and the micropylar one in the sac.

3. The embryo sac is the usual eight-nucleate kind, developed in the usual way.

4. Pollinia are in massulae in four loculi. Tetrads are in any position, but there is no rounding-up of pollen grains. Tube and generative nuclei are formed before the pollen escapes. The pollen tube shows a tube nucleus and two male nuclei as it enters the ovary.

5. Double fertilization occurs.

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EXPLANATION OF PLATES VII–IX

All figures were drawn with a Bausch and Lomb camera. *Fig. 8* was made with Leitz no. 4 ocular and no. 7 objective, and *fig. 36* with no. 2 ocular and no. 7 objective; all others with Leitz no. 4 ocular and Bausch and Lomb $\frac{1}{12}$ objective.

The abbreviations used are as follows: *c* chalazal daughter cell and nucleus; *e* egg; *g* generative nucleus; *gc* generative cell; *m* micropylar daughter nucleus; *ms* megaspore nucleus; *p* polar nucleus; *s* synergid; *t* tube nucleus; ♂ male nucleus.

PLATE VII

FIG. 1.—Young ovule.

FIG. 2.—Young ovule with broader funiculus.

FIG. 3.—Archesporial cell differentiated; beginning of the integument.

FIG. 4.—Mother cell; synapsis almost complete.

FIG. 5.—Synaptic knot complete; integument much older.

FIG. 6.—Metaphase; thirteen chromosomes; multipolar spindle.

FIG. 7.—Spindle for division of mother cell.

FIG. 8.—Mother cell in same stages as *fig.* 7, but integuments much farther advanced.

FIG. 9.—Late anaphase; no evidence of wall formation.

FIG. 10.—Late telophase; wall separating the daughter nuclei.

FIG. 11.—Two daughter cells.

FIG. 12.—Two daughter cells, one of which is disintegrating.

FIG. 13.—Two daughter cells in mitosis.

FIG. 14.—Two daughter cells in telophase with walls forming; these are the megaspores.

FIG. 15.—Two daughter nuclei with no wall separating them; traces of the spindle still present.

FIG. 16.—Two daughter nuclei with no separating wall, but one of them larger than the other.

FIG. 17.—Chalazal daughter cell in telophase, with no trace of wall; micropylar daughter cell with chromosomes formed for division.

FIG. 18.—Chalazal daughter cell in telophase, with beginning of wall formation; micropylar daughter cell shows a slight thickening of the spirem.

PLATE VIII

FIG. 19.—Chalazal daughter cell in prophase; spirem short and thick; micropylar daughter cell in late telophase with wall forming.

FIG. 20.—Two megaspore nuclei of equal size; micropylar daughter cell undivided.

FIG. 21.—Embryo sac beginning to increase in size; one megaspore nucleus beginning to show evidence of mitosis; another megaspore nucleus disintegrating within the sac; the two megaspore nuclei from the micropylar daughter cell also disintegrating, with slight traces of the spindle still present.

FIG. 22.—About the same stage as *fig.* 20.

FIG. 23.—Four megaspore nuclei, one from each of the daughter cells disintegrating with its own cytoplasm, the other one in each case in good condition for development, although the chalazal nucleus is the larger.

FIG. 24.—Micropylar daughter cell with chromosomes formed for mitosis; one megaspore nucleus in sac developing, the other already divided, and both nuclei disintegrating.

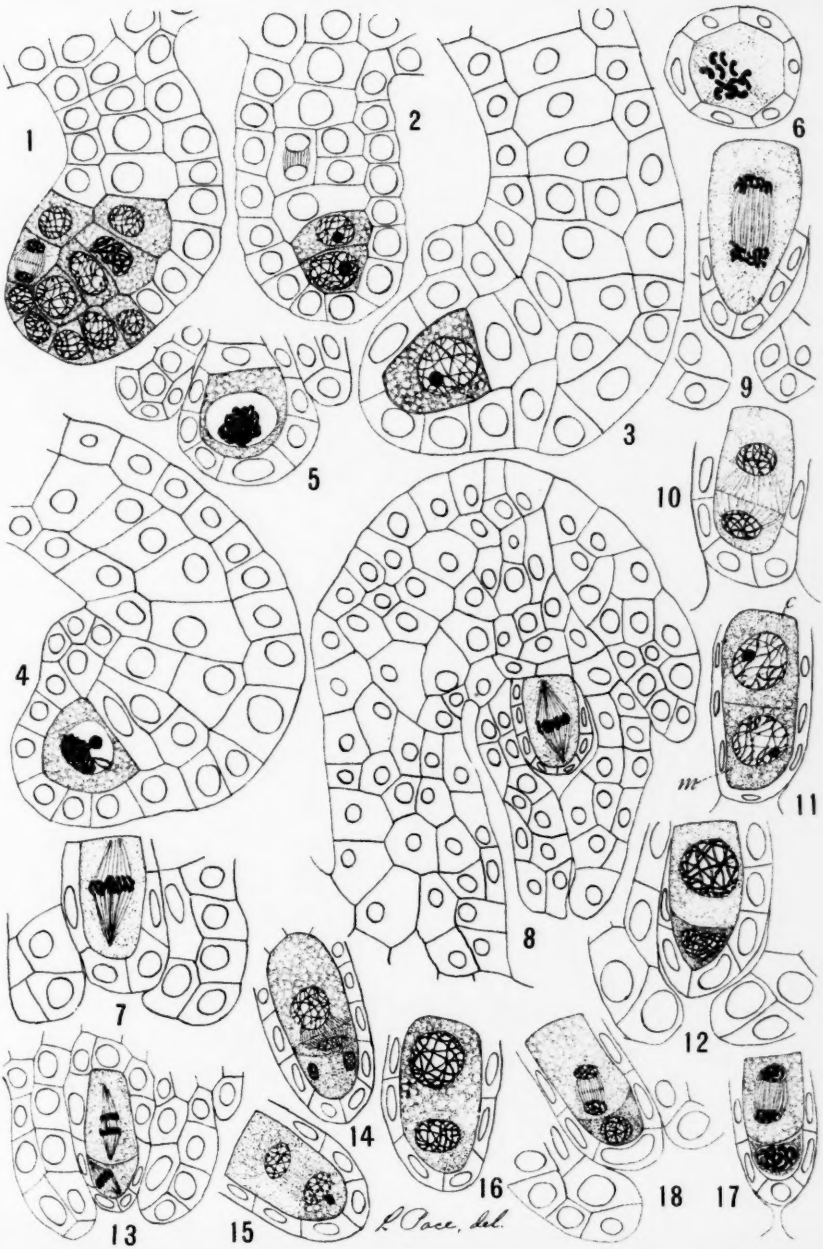
FIG. 25.—Chalazal megaspore nucleus with spindle; the other megaspore nucleus in the sac in late spirem, or metaphase; the micropylar daughter cell nucleus in spirem stage.

FIG. 26.—Two sporogenous cells in one ovule.

FIG. 27.—Two mother cells in one ovule.

FIG. 28.—Two mother cells with nucellar tissue between them.

FIG. 29.—Two mother cells.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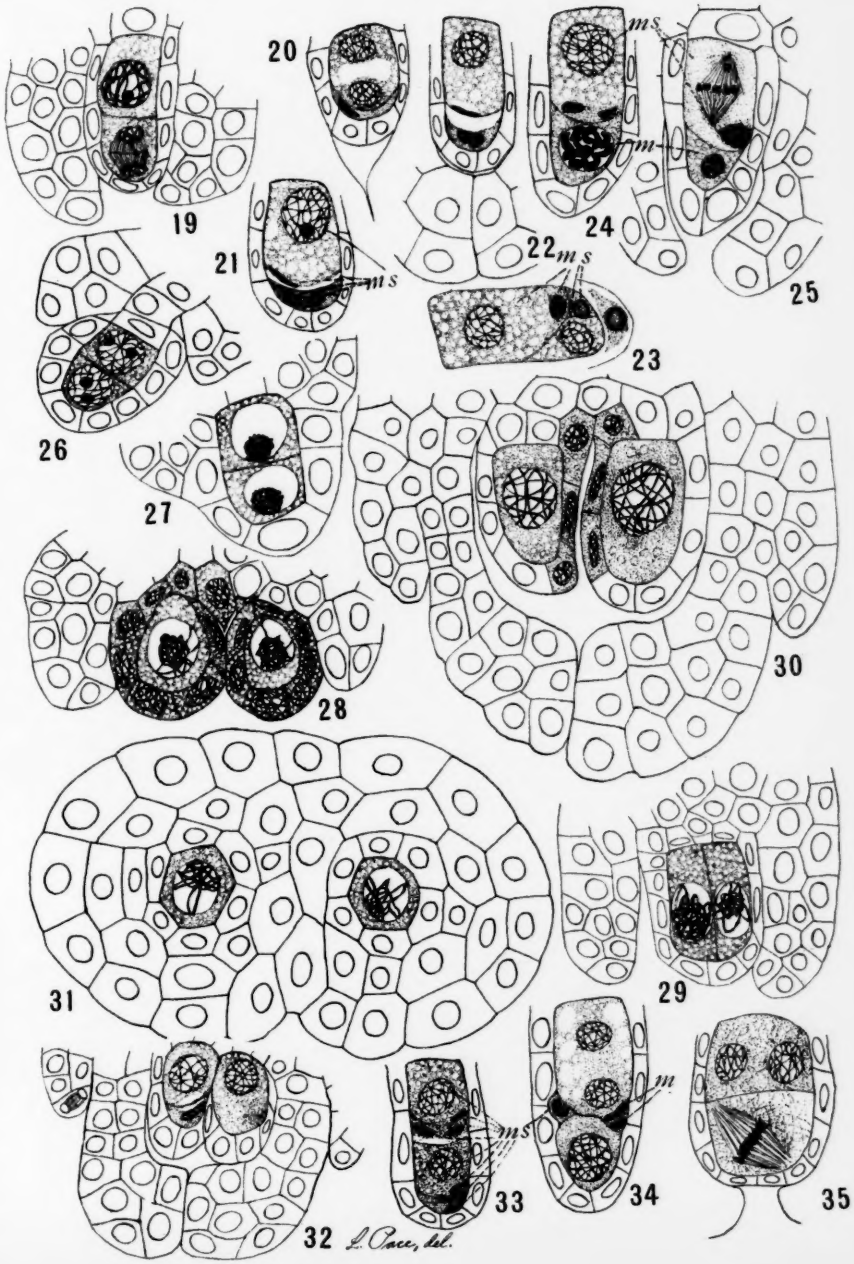
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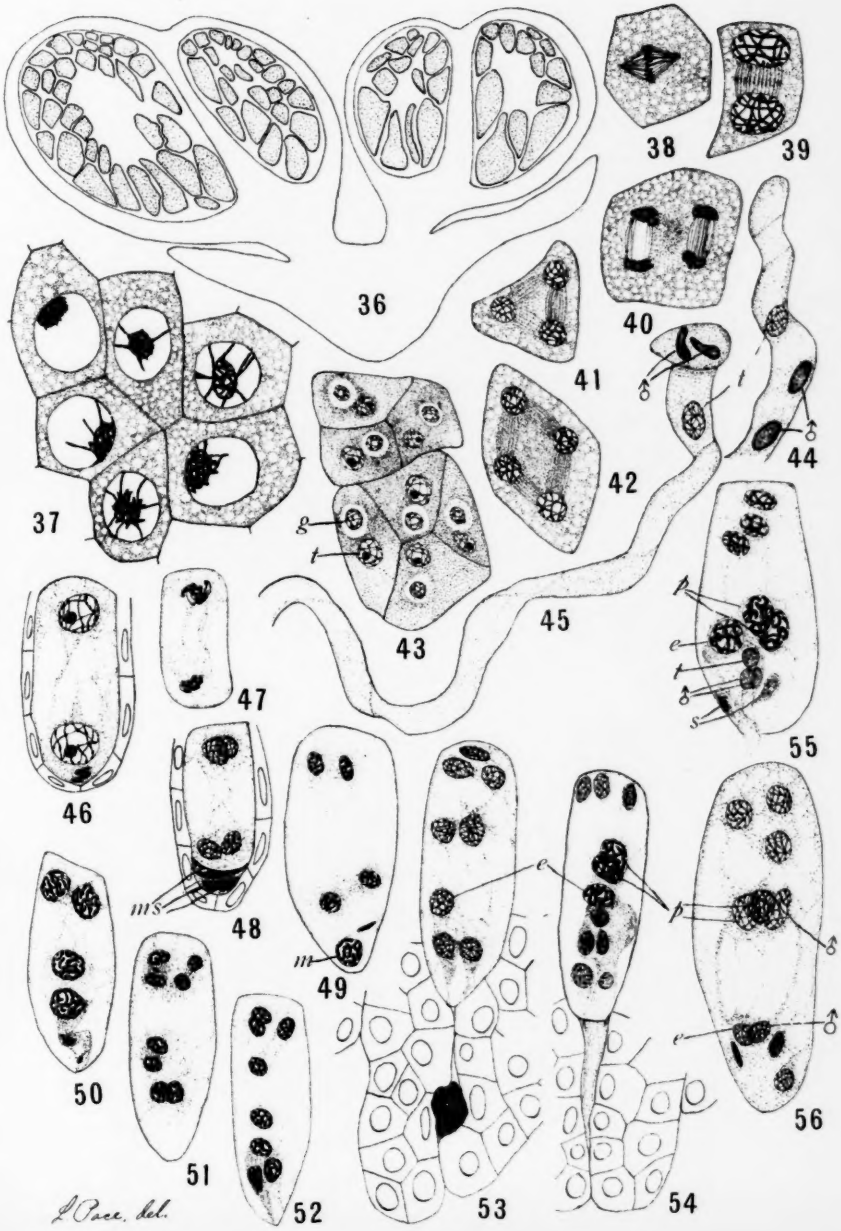
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FIG. 30.—Two mother cells after recovery from synapsis.

FIG. 31.—Two mother cells with several cells of nucellar tissue between; ovule cut across.

FIG. 32.—Ovule with two daughter cells; and probably another mother cell beside it, or another daughter cell with slight traces of the second disintegrating.

FIG. 33.—Eight megaspores from two mother cells.

FIG. 34.—Two-celled embryo sac, three disintegrating megaspore nuclei, and a mother cell recovered from synapsis.

FIG. 35.—One mother cell in metaphase, the other already divided, giving two daughter nuclei without separating wall.

PLATE IX

FIG. 36.—Diagram of anther, showing four pollen masses; massulae shaded.

FIG. 37.—Pollen mother cells in synapsis.

FIG. 38.—Pollen mother cell with spindle.

FIG. 39.—Pollen mother cell in telophase.

FIG. 40.—Telophase of second division.

FIGS. 41, 42.—Pollen tetrad; microspore nuclei.

FIG. 43.—Two tetrads, each pollen grain of which shows tube and generative nucleus.

FIG. 44.—Pollen tube from within ovary.

FIG. 45.—Pollen tube near ovule.

FIG. 46.—Two-celled embryo sac.

FIG. 47.—Both nuclei in sac in mitosis.

FIG. 48.—Four-celled embryo sac: three disintegrating megaspores.

FIG. 49.—Four-celled sac; traces of spindle of preceding division; megaspore nucleus within sac disintegrating.

FIG. 50.—Four-celled sac; prophase for next division; traces of disintegrating megaspore nucleus and micropylar daughter cell.

FIG. 51.—Eight-celled sac; traces of spindles of preceding division.

FIG. 52.—Nuclei beginning to assume usual position in sac.

FIG. 53.—Sac ready for fertilization; egg apparatus of egg and two synergids; two polars, one a sister to the egg; three antipodals; pollen tube between integuments.

FIG. 54.—Pollen tube within the sac; egg and two polars in late spirem or metaphase.

FIG. 55.—Pollen tube in sac; egg with thick spirem; polars with chromosomes.

FIG. 56.—Double fertilization.

ON MESARCH STRUCTURE IN LYCOPODIUM[1]

EDMUND W. SINNOTT

(WITH PLATE X)

Halftone

The position of the protoxylem in relation to the later-formed elements of the wood has been the subject of careful investigation in the various groups of vascular plants, both fossil and living, and has often been brought forward as evidence of the relationship of one group to another.

In his description of the anatomy of a specimen of Sigillaria, in 1839, BRONGNIART (**1**) showed the central cylinder to be formed of a ring of vascular bundles, each composed of an inner, primary part, and an outer, secondary part, the latter characterized by the radial arrangement of its elements. The small spiral elements lie on the outer edge of the primary wood, which was all developed in a centripetal direction, while the secondary wood grew centrifugally. The protoxylem is thus surrounded by later-formed elements.

In his classic work on the anatomy of the cycads, METTENIUS (**2**), in 1861, described the structure of the vascular bundle in the petiole and blade of certain living cycads. He showed that in the leaf-trace, as it leaves the cylinder of the stem, the protoxylem is all on the inside of the later-formed, mostly secondary, wood, but that as the bundle ascends into the petiole, the later-formed wood gradually bends around inwardly and incloses the spiral elements. Finally the centrifugal wood, which is probably all secondary, is greatly reduced, and separated from the protoxylem by parenchyma, while the centripetal wood forms the bulk of the bundle, and bears at its outermost point the cluster of spiral tracheids. METTENIUS (**2**, p. 582) noticed the resemblance between this bundle and one of the stem bundles of Sigillaria, and remarks that the much greater development of centrifugal secondary wood in the latter is due to the fact that it belongs to the vascular system of the stem.

RENAULT (**3**), however, was the first to really suggest the affinity

[1] Contributions from the Phanerogamic Laboratories of Harvard University, no. 16.

of the cycads with the Sigillariae on the basis of a common possession of this character. He carefully distinguished the irregularly arranged centripetal wood from the radially disposed centrifugal wood of the sigillarian bundle, and was the first to describe the structure of the leaf-trace in this genus. The elements in the leaf are also formed in two directions, those on the outside often being radially arranged. This structure making the comparison with the cycads still closer, RENAULT comes to the conclusion (**3,** p. 281) that "les Cycadées actuelles qui possèdent dans la structure du cordon foliaire cette analogie si frappante avec certaines plantes houillères, n'en sont que les représentants amoindris et en voie de décadence."

RENAULT has applied the term diploxylic to bundles possessing centrifugal as well as centripetal wood, and has grouped together Sigillaria and Poroxylon under the head DIPLOXYLÉES. Count SOLMS-LAUBACH (**4,** p. 263), however, called attention to the fact that where all the centrifugal wood is secondary, the original primary bundle, which thus has its oldest elements on the very outside, is of a different type from one where there is primary wood on the outside, as well as on the inside, of the protoxylem. For the former type of primary bundle he suggested the name exarch, and for the latter type mesarch, terms which have since been in current use with those meanings. RENAULT'S diploxylic implies the presence of secondary wood.

On the characteristic possession of one of these two main types of primary bundle, modern writers have often divided living and fossil vascular cryptogams into two great groups—the Fern series generally, as typically mesarch, and the Lycopodiales, fossil and living, as typically exarch.

This general rule, however, does not hold at all closely. Among living ferns, there are numerous instances of exarch structure, for example in the stem of *Trichomanes scandens*, of *Lygodium dichotomum*, and of *Loxoma Cunninghamii*. The same holds true of fossil ferns and their allies. Zygopteris shows both internal and external protoxylem, while Megaloxylon is clearly exarch.

Among the typically exarch forms, on the other hand, RENAULT (**3**) has figured mesarch leaf-traces in the Sigillariae. They are also found in certain species of Lepidodendron. Numerous cases of such development also occur in the modern relatives of this group. In

the center of each stem bundle of Tmesipteris there is a cluster of initial elements, the breaking-down of which often results in the formation of a lacuna. Although the cylinder of the stem of Selaginella is characteristically exarch, GIBSON (**5**) has noted, in the case of the protostelic *S. spinosa*, that in the trailing portion of the stem, all the protoxylem is in the center of the cylinder, completely inclosed by metaxylem. The root in the whole genus is also distinctly mesarch. In Phylloglossum, it is well known that the tubular stele at the base of the peduncle, the ring of bundles into which this divides higher up, and the traces of the sporophylls, have their first-formed wood elements surrounded on all sides by later ones. Exarch development seems entirely lacking, both in this genus and in Tmesipteris.

In Lycopodium, however, precisely the reverse seems at first sight to be true, and I have been unable to find a recorded instance of the occurrence of mesarch development in the genus.

In an endeavor to determine the presence or absence of such a character, the following species and varieties of Lycopodium were investigated: *L. inundatum* L., and var. *Bigelovii* Tuckerm.; *L. lucidulum* Michx.; *L. annotinum* L.; *L. obscurum* L.; *L. tristachyum* Pursh; *L. complanatum* L. var. *flabelliforme* Fernald; and *L. clavatum* L.

L. inundatum and its variety are somewhat delicate forms, and have by far the most poorly developed vascular system of the species looked at. In both, the xylem rays are few in number (3–6), and along the much broadened end of each extends a row of crushed protoxylem elements. There is no indication of metaxylem outside of these. In the leaf-trace, however, especially at a little distance from the cylinder, the smaller elements, ringed or loosely spiral, tend to become clustered at the center of the bundle. Just before the trace enters the leaf, these elements break down, leaving a protoxylem lacuna, completely surrounded by later-formed, closely spiral elements. This is perfectly evident in both transverse and radial sections (*figs. 1, 2*).

In the case of *L. lucidulum*, the central cylinder is also small, in comparison to the diameter of the stem, and its xylem rays are few in number and broadened at the ends. As in *L. inundatum*, however, there is no indication of centrifugal wood in the cylinder itself, but the

leaf-traces, though composed of a comparatively small number of cells, show in nearly every case a mesarch structure (*fig. 3*).

The remaining species studied have much better developed vascular tissues. Their cylinders are in general very similar, being composed of a much larger number of xylem rays, which end more or less acutely. In all the species, metaxylem cells occur just outside the protoxylem of the cylinder. In *L. tristachyum* and *L. complanatum* var. *flabelliforme*, this mesarch development is not common, but in *L. obscurum* and *L. clavatum*, especially the latter, it is very noticeable. In vertical section, the centrifugal elements are seen to be reticulate, not scalariform, as in the rest of the metaxylem (*figs. 4, 6*). The exceptional development of this structure in *L. clavatum* may be due to the fact that its vegetative growth is more luxuriant than that of any of the other species studied. In all these forms, the leaf-trace again is very clearly mesarch (*figs. 7–9*). This is especially conspicuous, perhaps, in *L. tristachyum* and *L. complanatum* var. *flabelliforme*, where a large protoxylem lacuna occurs in the center of the trace (*fig. 9*).

In all the species, the leaf-trace, as it leaves the cylinder, is very small, but rapidly increases in size in the cortex, where it possibly serves to store water.

The development of the protoxylem of the central cylinder is always from without inward. The single row of centrifugal metaxylem elements occurs directly outside the earliest-formed xylem cells. Sections through the growing-point of *L. clavatum* showed that the metaxylem elements on both sides of the protoxylem developed nearly simultaneously. In the leaf-trace, also, as was shown by a very young stem of *L. complanatum* var. *flabelliforme*, the elements surrounding the protoxylem are all developed at the same time.

The leaf-trace of *Selaginella rupestris* (L.) Spring was investigated, and though composed of a very small number of cells, it showed a strong tendency toward mesarch arrangement. It is interesting to note here an observation of GIBSON (**6**, p. 151) on the leaf bundle of Selaginella. "In the upper third of the leaf the protoxylem elements become accompanied by several short reticulate tracheides which flank the spiral tracheides, and in section (e. g., of *S. Braunii*) may inclose the spiral elements completely."

It is noteworthy that in certain of the fossil Lycopodiales, as above mentioned, there is clear evidence of the possession of mesarch structure in the primary wood of the leaf-trace.

That there is no sharp line of cleavage between mesarch and exarch vascular cryptogams is thus perfectly clear. Among the main groups, we have numerous instances of both types of development, and the one merges, little by little, into the other. In Lycopodium, the centrifugal wood of the stem, when present, consists of only a few tracheids; in such forms as Lyginodendron, for instance, and in many ferns, the spiral elements are near the outside, while in Tmesipteris and Phylloglossum, they are in the very center. The variability of the position of the protoxylem in the primary wood bundle, and its consequent unreliability as a phylogenetic character, cannot be too strongly emphasized.

While the structure of the primary bundles of the vascular cryptogams cannot be used as a guide to their interrelationships, it furnishes an excellent character for the whole group, both living and fossil forms. The presence in the stem of *centripetal primary wood, continuous with the protoxylem*, is the distinguishing mark of these plants. The older French botanists appear to have been perfectly right in characterizing their *bois centripète* as cryptogamic wood, for the Sphenophyllales, Lycopodiales, and Filicales show this structure very clearly. The only apparent exception[2] to the rule is the calamitean series, including the modern Equisetales. A species of Calamites, however, the so-called *C. pettycurensis*, or Protocalamites, has recently been shown to have well-developed centripetal wood on the inner face of the protoxylem canals of the stem (SCOTT **7**). Further, EAMES (**10**), working in this laboratory, has distinguished a mesarch condition, with consequent presence of centripetal xylem, in the traces of the vegetative and reproductive leaves of several living species of Equisetum. It thus seems certain that centripetal wood was once well developed in the Calamites and their allies, but in the process of time has been almost entirely lost.

It is worthy of note that though secondary wood is often present in the vascular cryptogams, and may constitute the bulk of the central cylinder, the protoxylem is intimately associated with the centrip-

[2] Exclusive of that aberrant group of ferns, the Ophioglossales.

etal primary wood. In the higher plants, the centrifugal xylem preponderates, and the protoxylem becomes continuous with it, even in the cases where centripetal elements are present. The only exceptions to this rule occur in foliar bundles. The leaves of living cycads and of the Cordiates show true cryptogamic wood in their vascular strands, with the protoxylem closely associated with it, though in all cases centrifugal secondary wood is present. This persistence of a cryptogamic structure in the leaf, among the higher plants, furnishes good evidence of the conservatism of the foliar bundle. Scattered centripetal elements appear in the peduncle of certain cycads and in the cotyledons of Ginkgo, also seats of vestigial characters, and centripetal wood is fairly well developed in the leaf of Prepinus (JEFFREY **9**) and in the stems of many of the Cycadofilices; but in all these cases, the bundle is of the higher type, the protoxylem being continuous with the *centrifugal* wood.

The structure of the vegetative stem, of the floral axis, and of the leaf, in certain living Cycadaceae, shows clearly the transition from the lower type of wood to the higher. In the stem, the protoxylem joins the centrifugal wood and there are no centripetal tracheids. In the stalk of the male cone of *Stangeria paradoxa*, and to a less extent in the male and female peduncles of three other species of cycads, as shown by SCOTT (**8**), scattering centripetal xylem elements are present. They are not connected with the protoxylem, however, but are separated by parenchyma from the rest of the bundle, which otherwise is exactly similar to those found in the stem. In the petiole and blade of the cycad leaf, however, there is more than this faint suggestion of ancestral structures; for here, as above described, true cryptogamic wood occurs, consisting of well-developed centripetal elements directly continuous with the protoxylem. In the petiole, and sometimes in the blade, centrifugal wood is present as well. This is separated by parenchyma from the protoxylem, and appears to be largely, if not entirely, laid down by cambial activity. Its resemblance to a sigillarian bundle, as noted by RENAULT, is thus pretty exact. SCOTT, however, believes that some, at least, of the centrifugal wood is primary, and consequently that true mesarch development takes place, a condition which he compares to that found in the stem bundles of *Lyginodendron Oldhamium*. He presents the mesarch structure

of the peduncle and of the leaf as evidence of the affinity of modern cycads to the fossil Lyginodendreae and Poroxyleae. While the conclusion as to affinities is very probably correct, the argument is not entirely convincing. The cryptogamic ancestry of the cycads is proven, but that is all; for we have already seen that the possession of mesarch primary wood is limited to no one group of plants, and that its presence or absence is of no value as phylogenetic evidence.

CONCLUSIONS

The position of the protoxylem in the primary wood bundle of vascular cryptogams is very variable, for in all of the groups it may be almost anywhere in the strand. It is consequently of little value in determining the relationship of any group to other groups or to the higher plants. A constant primitive feature in the vascular cryptogams, however, is the presence in the stem of cryptogamic wood—centripetal xylem continuous with the protoxylem elements. Whenever such a feature occurs in any of the higher plants, it can be used as evidence in tracing their affinity only with vascular cryptogams in general, not with any particular group of them.

I desire to express my thanks to Dr. M. A. CHRYSLER and to Mr. A. J. EAMES for assistance in procuring material, and especially to Professor E. C. JEFFREY for advice during the course of the work.

This investigation was carried on in the Phanerogamic Laboratories of Harvard University.

HYANNIS, MASS.

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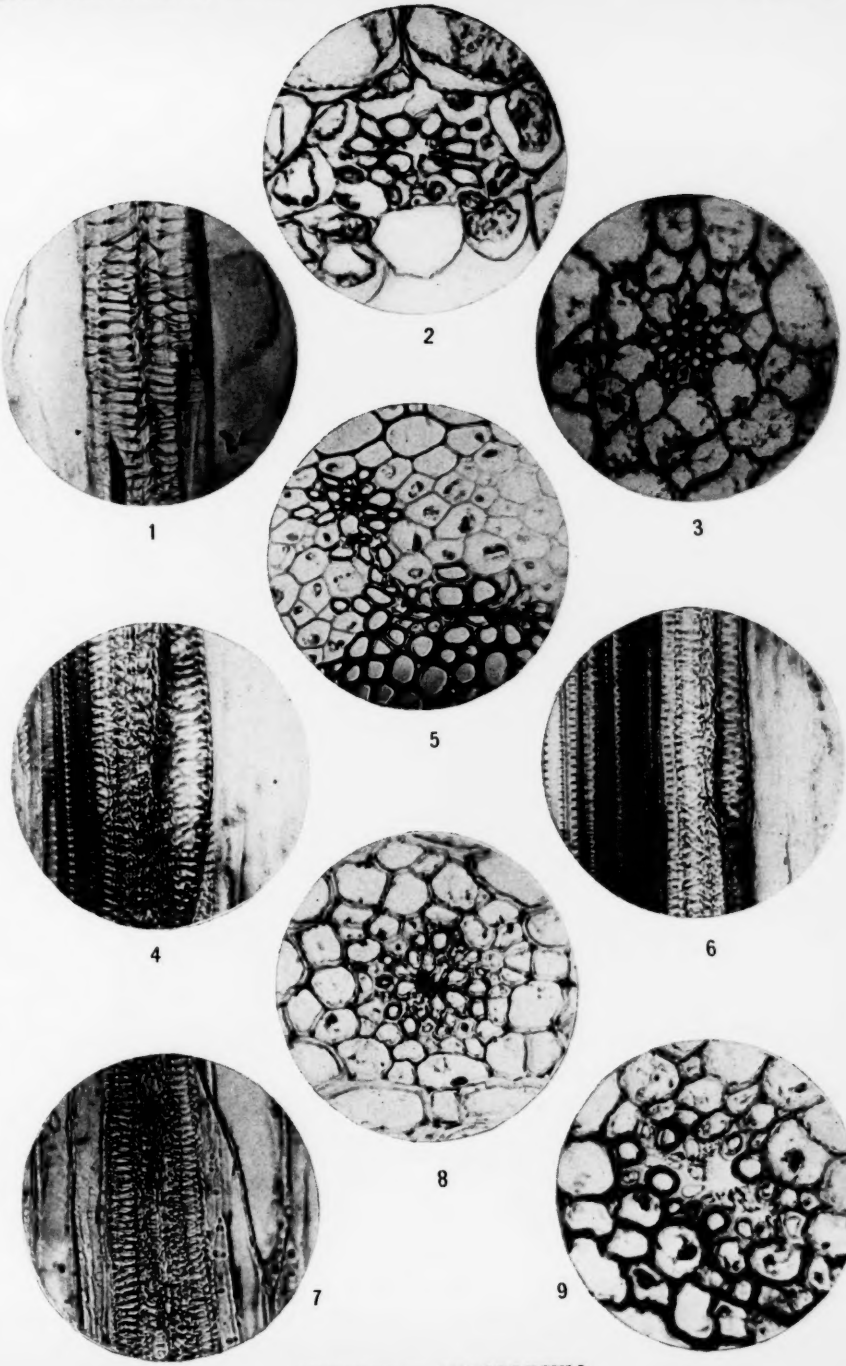

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EXPLANATION OF PLATE X

Fig. 1.—*Lycopodium inundatum* var. *Bigelovii;* longitudinal radial section through a leaf-trace. ×500.

Fig. 2.—*Lycopodium inundatum* var. *Bigelovii;* transverse section of a leaf-trace near the margin of a mucilage cavity. ×500.

Fig. 3.—*Lycopodium lucidulum;* transverse section of a leaf-trace. ×500.

Fig. 4.—*Lycopodium clavatum,* longitudinal radial section through the margin of the central cylinder, showing the single centrifugal metaxylem element on the right. ×500.

Fig. 5.—*Lycopodium clavatum;* transverse section through the margin of the central cylinder at the point of departure of a leaf-trace; the dark line of crushed cells marks the position of the protoxylem. ×500.

Fig. 6.—*Lycopodium obscurum;* longitudinal radial section through the margin of the central cylinder, showing centrifugal metaxylem as in *fig. 4.* ×500.

Fig. 7.—*Lycopodium clavatum;* longitudinal radial section through a leaf-trace. ×500.

Fig. 8.—*Lycopodium clavatum;* transverse section of a leaf-trace. ×500.

Fig. 9.—*Lycopodium tristachyum;* transverse section of a leaf-trace. ×500.

BRIEFER ARTICLES

SOME HITHERTO UNDESCRIBED PLANTS FROM OREGON

Mr. WILLIAM C. CUSICK has submitted to the writer for determination an interesting series of castillejas and senecios, collected by him during the past few years in the Wallowa Mountains of northeastern Oregon. The material is copious and for the most part falls readily into well-known species. There are, however, a few plants which do not satisfactorily accord with any described species; these may be here characterized as follows:

Castilleja chrysantha, n. sp.—Herbacea perennis tota planta glanduloso-villosa: caulibus erectis vel ascendentibus 0.5–3^{dm} altis: foliis linearilanceolatis vel lanceolatis vel interdum subovatis 1–3^{cm} longis 1.5–10^{mm} latis integris vel trifidis trinerviis crispo-hirsuto-glandulosis, laciniis linearibus acutis patentibus; inflorescentiis spicatis 1.5 to 14^{dm} longis; bracteis anguste lanceolatis vel lanceolatis integris vel trifidis flavo-viridibus vel non rarius paululo purpurascentibus; calyce 13–18^{mm} longo antice et postice subaequaliter fisso ad circiter medio altitudinem connato, laciniis oblongis et integris ad apicem rotundatis vel bilobatis, lobis oblongis obtusis exteriore glanduloso-villosis viridibus vel aureoviridibus vel rarius paululo purpurascentibus; corolla 16–20^{mm} longa flava; galea recta 4–6^{mm} longa; labium 2.5–3^{mm} longum, labii lobis oblongo-ovatis obtusis calycem superantibus; capsula oblongo-elliptica circiter 8^{mm} longa breviter acuminata glabra.

In wet meadows at the head of West Eagle Creek, Wallowa Mountains, Oregon, altitude 2135^{m}, 15 August, 1907, *William C. Cusick*, no. 3200^{b} (type hb. Field Mus. Cat. no. 225021); in moist soil near the source of the Imnaha, Wallowa Mountains, altitude 2440^{m}, 14 August, 1906, *William C. Cusick*, no. 3124 (hb. Field Mus.); on dry mountain sides, Kettle Creek, Oregon, altitude 1830^{m}, July, 1906, *William C. Cusick*, no. 3103^{a} (hb. Field Mus.); in wet meadows, Wallowa Mountains, Oregon, altitude 2135^{m}, 19 August, 1907, *William C. Cusick*, no. 3205^{a} (hb. Field Mus.); source of the Wallowa River, altitude 2440^{m}, 15 August, 1908, *William C. Cusick*, no. 3324^{c} (hb. Field Mus.); wet subalpine prairie of the Wallowa Mountains, 28 August, 1898, *William C. Cusick*, no. 2110 (hb. Field Mus.), distributed as "*Castilleia oreopola* Greenman, white-flowered form."

The habit and glandular pubescence of this species suggests a close relation ship to *C. viscidula* Gray and *C. Covilleana* Henderson. From the former *C. chrysantha* differs in having a longer calyx with oblong and rounded or obtuse

instead of acute ultimate calyx divisions; the lip is longer in proportion to the galea, and the pubescence is more villous. From *C. Covilleana* it differs in the less deeply cleft bracts, in the oblong blunt divisions of the calyx, and in the longer exserted lip of the corolla.

Castilleja fraterna, n. sp.—Herbacea perennis; caulibus caespitosis numerosis ascendentibus plus minusve flexuosis 0.5–1.5^{dm} altis viridibus vel purpurascentibus in partibus inferioribus glabris, superioribus villosis hinc inde glanduloso-pubescentibus; foliis linearibus vel lanceolatis 1–2.5^{cm} longis integris vel trifidis pubescentibus, laciniis linearibus acutis patentibus; spicis pauci-multifloris 5^{cm} vel minus longis; bracteis plerumque trifidis rubris; calyce circiter 2^{cm} longo exteriore pubescente antice quam postice fissiore laciniis lateralibus 2-lobatis, lobis lanceolato-oblongis obtusis rubris; corollis 2.5–3^{cm} longis exteriore plus minusve pilosis flavis et rubellis; galea erecta circiter triplo brevior quam tubus flava vel flavo-viridia, margine rubello, labio 2.5–3.5^{mm} longo basi triplicato plerumque atro-viridi, labii lobis anguste-ovatis acutis vel obtusis ad apicem rubicundis; capsula oblonga 8–10^{mm} longa brevi-acuminata acuta glabra.

In alpine regions of the Wallowa Mountains, Oregon, 14 August, 1906, *William C. Cusick*, no. 3125 (type, hb. Field Mus. Cat. no. 225016); and in the same locality, 27 August, 1907, *William C. Cusick*, no. 3222° (hb. Field Mus.).

Mr. CUSICK states that the plants here cited were associated with Salix, and that they grew especially in thickets of *S. sitchensis*. The numerous subflexous stems, ascending from a common base, each terminated by a conspicuous inflorescence variegated with green, red, and yellow, render it an extremely attractive species.

Castilleja oresbia, n. sp.—Herbacea perennis basi lignosa; caulibus simplicibus erectis vel ascendentibus 1.5–2^{dm} altis crispo-hirsutis; foliis lineari-lanceolatis et integris vel trifidis 1–4^{cm} longis 2–5^{mm} latis crispo-pubescentibus, laciniis linearibus acutis patentibus; inflorescentiis terminalibus dense spicatis 1.5–4.5^{cm} longis; bracteis saepissime trifidis, lobis lateralibus linearibus obtusiusculis, lobo intermedio majore lanceolato-ovato; calyce 10–14 ^{mm} longo exteriore pubescente antice et postice aequaliter fisso, lobis oblongis bidentatis; corolla flava 14–17^{mm} longa glabra, galea recta circiter 6^{mm} longa, labii lobis lineari-elongatis 4^{mm} longis acutis; capsula oblonga acuta circiter 1^{cm} longa glabra.

On dry mountain sides, Kettle Creek, Oregon, altitude 1830^{m}, 19 August, 1907, *William C. Cusick*, no. 3201^{a} (hb. Field Mus. Cat. no. 225022).

In habit and general appearance this species resembles most closely *C. rustica* Piper, from which it differs in having shorter, more densely flowered, and less villous spikes, and in the characters of the corolla. A marked distinguishing

feature of *C. oresbia* is the straight corolla with the conspicuously long lobes of the lower lip.

SENECIO HOWELLII Greene, var. **lithophilus,** n. var.—A forma typica recedit foliis multo amplioribus, maximis 3–15^{cm} longis 1–3.5^{cm} latis integris vel inaequaliter et alte dentatis.

In stony dry soil, Imnaha, Wallowa Mountains, Oregon, about 1830^{m}, 12 August, 1906, *William C. Cusick*, no. 3129 (hb. Field Mus.).

Mr. CUSICK's specimens under the number here cited seem at first glance to be more than of varietal significance, but a comparison with the type of *S. Howellii* and a considerable suite of specimens from the Gray Herbarium and in the herbarium of the Field Museum show the plant in hand to be only an extreme variation with large, broad, entire or coarsely and unequally dentate leaves.—J. M. GREENMAN, *Field Museum of Natural History, Chicago.*

CURRENT LITERATURE

BOOK REVIEWS

Ecology of plants

For a dozen years English and American botanists have been more or less hopefully awaiting a translation of WARMING'S *Plantesamfund*, which was promptly translated into German and thus made available to a larger audience. A peculiar accumulation of misfortunes of one kind or another prevented an English edition of this epoch-making work, but at last WARMING'S contributions are made available to all English and American botanists, and in the most happy way possible, through the preparation of an essentially new book.[1] The author has excellent command of the English language, having frequently contributed articles in this tongue. The new volume was written in English by the author himself, assisted by Dr. MARTIN VAHL, and was prepared for publication by Drs. PERCY GROOM and I. B. BALFOUR. A preface written by the author calls attention to the more fundamental changes in the English work; these are so many and so important that an extended review is necessary.

The introduction contains considerable new matter concerning growth forms (*Lebensformen* or *Vegetationsformen*), together with a new and rather satisfactory classification of them. There are six categories of growth forms: heterotrophic, aquatic, muscoid, lichenoid, lianoid, and all other autonomous land forms. The final category is, of course, much the largest, and is subdivided into monocarpic and polycarpic forms. Monocarpic growth forms may be aestival annuals, hibernal annuals, or biennials to perennials. The polycarpic forms are much more numerous, and their subdivision is based largely on the idea of the character of their protection during the severest seasons, taking account of such things as the duration of the vegetative shoot, the length and direction of the internodes, the position of the renewal buds, the bud structure, size, leaf duration, and the adaptation of the nutritive shoot to transpiration. The sub-classes of polycarpic forms are renascent plants (subdivided into plants with multicipital rhizomes, mat geophytes, and traveling or rhizome geophytes), rosette plants (subdivided into ordinary rosettes and tree rosettes), creeping plants, and plants with erect long-lived shoots (subdivided into cushion plants, undershrubs, soft-stemmed plants, succulent-stemmed plants, and woody plants with long-lived lignified stems).

[1] WARMING, EUG., assisted by MARTIN VAHL, Oecology of plants; an introduction to the study of plant communities. Prepared for publication in English by PERCY GROOM and ISAAC BAYLEY BALFOUR. Royal 8vo. pp. xii+424. Oxford: Clarendon Press. 1909. Cloth, $2.90; morocco, $3.25.

The first and second sections, dealing with ecological factors and their action and the communal life of organisms, are but little changed, except that they are brought up to date, as is true of every part of the book to a most remarkable degree. Sun and shade plants are called heliophytes and sciophytes respectively, and their leaves are denominated heliophylls and sciophylls. The third section brings together the parts referring to the adaptations of land and water plants, distributed under various heads in the Danish and German editions. Much more vital, however, involving new conceptions rather than rearrangement of material, is the new classification of plant formations. Here the author departs widely from his former fourfold classification of plants into hydrophytes, mesophytes, xerophytes, and halophytes. The new conception is that there is a fundamental twofold subdivision into land plants and water plants. The land plants are subdivided further into twelve primary groups, thus making with the hydrophytes thirteen main classes of plant formations, in place of the four classes of previous editions. For the new classification, the author states that Dr. VAHL is largely responsible, and especially for those following the psammophytes, as noted below.

In former editions it will be recalled that WARMING objected, and with good reason, to the word formation as an ecological unit, largely because of its varied use by different authors. But language is a peculiar thing, and ill-chosen words often stick. It has been so with formation, and the author now attempts to delimit the word, regarding a formation as "a community of species, all belonging to definite growth forms, which have become associated together by definite external characters of the habitat to which they are adapted." The chief classes of formations are microphyte, moss, herb, undershrub, shrub, and forest, and individual formations may be simple, compound, or mixed. An association is defined as "a community of definite floristic composition within a formation, a floristic species of a formation which is an oecological genus." The conception of a formation as an ecological genus and an association as an ecological species is now becoming generally accepted in principle, but this concrete statement by the father of modern ecology should make its acceptance universal.

The new classification of formations, with some of the leading sub-classes under each, follows.

A. Soil wet; water available. (1) Hydrophytes; subdivided into plankton (further split up into haloplankton, limnoplankton, and saproplankton), cryoplankton (including the microphytes of ice and snow), hydrocharid formations or pleuston, the lithophilous benthos (in place of the nereids), the benthos of loose soil (including hot spring microphytes, sand algae, saprophytic microphytes, and the enhalid and limnaea formations, as in previous editions). (2) Helophytes or swamp plants; subdivided into reed swamps and bush swamps.

B. Soil physiologically dry. (3) Oxylophytes or plants of sour (i. e., acid) soil, characterized largely by xeromorphy; subdivided into low moors, grass heaths, high moors, moss and lichen heaths (or tundra), dwarf shrub heaths, and bushland or forest on acid soil. (4) Psychrophytes or plants of cold soil,

including chiefly the subglacial fell-fields. (5) Halophytes; subdivided into lithophilous, psammophilous, and pelophilous halophytes, salt swamps and deserts, and littoral swamp forests (mangrove swamps).

C. Soil physically dry and dominant in determining the vegetation. (6) Lithophytes; subdivided into the true lithophytes (chiefly lichens) chasmophytes, and shingle and rubble plants. (7) Psammophytes or sand plants. (8) Chersophytes or waste plants (i. e., ruderals); subdivided into waste herbage and bushland on dry soil.

D. Climate dry and dominant in determining the vegetation. (9) Eremophytes, or plants of steppes and deserts; subdivided into deserts, shrub steppes, and grass steppes (including prairies). (10) Psilophytes or savanna plants; subdivided into thorny savanna, true savanna, and savanna forest. (11) Sclerophyllous plants; subdivided into garique, maqui, and sclerophyllous forest.

E. Soil physically or physiologically dry. (12) Conifers.

F. Soil and climate favorable to mesophilous formations. (13) Mesophytes; subdivided into arctic and alpine mat grassland, meadow, pasture, mesophytic bushland, deciduous dicotylous forests, and evergreen dicotylous forests.

The classification here outlined does not strike the reviewer as an improvement over the one abandoned. For the most part the new groups of land formations are segregates from the old term xerophyte. It is true that the latter term had become unwieldy, but it is questionable whether the difficulties are solved. Among the advantages of the new scheme is the recognition of close relationship between heath and moor plants, which together form the category oxylophytes, and also the close ecological relationship between these plants and the plants of cold and salty soils, as emphasized by SCHIMPER. It is gainful, too, to put the edaphic xerophytes (lithophytes, psammophytes, chersophytes) into one category and climatic xerophytes (eremophytes, psilophytes, sclerophylls) into another. Among the disadvantages of the new arrangement are many instances where unrelated things are placed together and related things are separated. The most conspicuous case of the former is seen in class 12, the conifers. While this group is a floristic unit, and even an ecological unit from the anatomical standpoint, it is far from being a geographic unit of any sort. It would seem better to put many conifers with the lithophytes and psammophytes, while others are certainly oxylophytes, and others still pronounced mesophytes, the most mesophytic forests of the United States being dominated by conifers. Again, classing the plants of steppes and deserts together as eremophytes does an injustice to the American prairie, much of which is essentially mesophytic. Repetitions in listing the plant formations of a region would be certain to occur, for example as between lithophytes and psammophytes, oxylophytes and psychrophytes in northern regions, etc. It also seems unfortunate that so many Greek derivatives are employed. Nowadays we are getting more and more to abandon the classics and their lengthy derivatives for the vigorous and familiar terms of the vernacular, and it seems a pity to have to brush the dust once more from our Greek lexicons. The antiquated spelling of ecology (oecology) is unfortunate, and is

made more striking by contrast with the frequently used economy, which has the same reason for appearing as oeconomy.

While words of adverse criticism seem necessary here and there, one may write volumes of praise. WARMING'S *Plantesamfund* will be for all time the great ecological classic, and the English volume now before us is the most important ecological work in any language. It is at the same time an old book and a new, a translation of the masterpiece of 1895 and a compendium of the ecological thought of 1909. WARMING has been contributing to ecology for more than forty years, and is the undisputed Nestor of the subject, but unlike many a Nestor, WARMING incarnates the ambitions and plasticity of youth. It will be pleasing to American ecologists to see the remarkable recognition accorded to their work in this new volume. The German edition of 1896 contained but one American title, though a half-dozen more might have been included. Now there are 600 titles in all, almost exactly twice the number published in 1896, and it is not unfair to say that half of the ecological work thus far accomplished is represented by the added titles of the last thirteen years. It will be flattering to Americans to note that 115 of the 300 new titles represent American contributions, a record that measures up well with the bare half-dozen that might have been named in the original 300. The new edition has ample footnote references, adding inestimably to the service of the work. The absence of illustrations will be a source of disappointment to many, but it accounts in large part for the extremely low price of the volume, a price that will insure a sale that has been accorded to no ecological work in the English language.—HENRY C. COWLES.

Experimental morphology

Although experimental morphology received its original impetus from observations on plants, during the last few years there has been a great dearth of botanical contributions to this field as compared with what the zoologists have done. It is partly with the hope of stimulating anew botanical exploration in this fascinating field that GOEBEL has published a course of lectures which he delivered in 1906–1907.[2] The field of experimental morphology, perhaps from necessity, is arbitrarily limited to what is often called developmental physiology, no reference being made to the direct influence of gravity, light, etc., on form. The author refers those who wish information on such topics to PFEFFER'S *Physiology* and his own *Organography*. Furthermore, the material presented is mainly from the higher plants, reference being made to KLEBS for similar material on the lower forms.

Especially interesting is the first chapter, which considers the mission of experimental morphology, for here the author reveals his philosophy. It is clear that GOEBEL goes about as far as KLEBS in referring plant phenomena to the influence of external factors, and he follows KLEBS in holding that the ordinary

[2] GOEBEL, K., Einleitung in die experimentelle Morphologie der Pflanzen. 8vo. pp. vi + 260. *figs. 135*. Leipzig and Berlin: B. G. Teubner. 1908. *M* 8.

succession of events in the life-history of a plant is no more normal than any other succession of events that may be produced by altering the external conditions. Like KLEBS, therefore, he is obliged to discard the word normal or place it in quotation marks. To the reviewer such a viewpoint seems fundamentally sound, and it has the added attraction of throwing open to experimentation all the phenomena of development, including those that have been referred to heredity or mysterious internal causes. In harmony with this fundamental principle, illustrations are given of the omission of individual stages from a developmental series, the transposition of two such stages, and the retention of a given stage, if the conditions favorable to another are not forthcoming; even the juvenile stages may be retained through life in many instances, if the external conditions are favorable thereto. On the other hand, there are cases where juvenile forms bear flowers, the intervening stages being skipped.

The body of the book consists of four chapters, dealing respectively with the influence of external and internal conditions on leaf form, the conditions determining variations in the development of main and lateral axes, regeneration, and polarity. In all of these chapters there is a wealth of detail of the utmost value to students in experimental morphology and to those desiring a bibliography of these subjects. Not only is the literature summarized, but there are many new and suggestive experiments mentioned here for the first time. In the chapter on leaf form, heterophylly in xerophytic species of Veronica and in such amphibious plants as Limnophila plays an important part. Mutation in some ferns is definitely referred to the operation of external factors. Plants with orthotropous and plagiotropous shoots are found to belong to two categories. For example, lateral branches of the spruce (*Picea excelsa*) become orthotropous if the terminal shoot is removed, while such a reaction does not occur in Araucaria; the former illustrates lability, the latter stability. The same chapter contains some very interesting data concerning variation in flowers and inflorescences. Of especial significance is the transformation of Selaginella "flowers" into vegetative shoots by introducing the plants into moist air. Variations in flower color and form, and the relations of cleistogamous and chasmogamous flowers are here considered, much credit being properly given to KLEBS for his path-breaking work. The chapters on regeneration and polarity are most useful in gathering together the scattered literature, as well as in adding new facts.

Although the volume is denominated an introduction and not a handbook, it is indispensable not only to experimental morphologists, bnt to all morphologists, physiologists, and ecologists, who are at all interested in the origin and significance of plant structures. To the reviewer the book is a bit disappointing, because it seems not to get down to fundamental principles. Throughout the the work nutrition is regarded as a dominant external factor. While it is true that it is as yet impossible to resolve nutrition into its components, it is at least better to attempt to do so than to assume that nutrition is really anything definite, or even ultimate. To many of us the work will be more useful as a summary of facts than as an explanation of the conditions underlying them.—HENRY C. COWLES.

The vegetation of Switzerland

CHRIST'S *Das Pflanzenleben der Schweiz* was published in 1879. A French translation of this work entitled *La flore de la Suisse et ses origines* appeared in 1907.[3] An apology is due the author and publishers for the tardiness of this review. The translator is E. TIÈCHE, and the author has added a supplement in which he summarizes the geobotanical work that has been done since the publication of the first edition. As the original work has never been reviewed in the GAZETTE, a summary of its contents may be acceptable.

After a brief discussion of the fundamental principles of plant distribution, especially the influence of climate, and migrations, the vegetational regions of Switzerland are described in great detail. The primary divisions are naturally zonal in form, since the country is so largely mountainous. The author enumerrates four zones: (1) the basal zone, having considerable likeness in its flora to the Mediterranean region, and largely under cultivation, with the grape as the characteristic culture plant; (2) the zone of deciduous forest, dominated in the south by the chestnut, in the north by the beech; (3) the zone of coniferous forest, composed of spruce, fir, larch, and Cembran pine; (4) the alpine region, to which a third of the discussion is devoted. Here particularly the author considers the problems of migration, tracing an important element among the alpine plants to the mountains of north-central Asia as their point of origin. The large endemic element of the alpine flora is also discussed at some length.

The work is characterized by minuteness of detail, possible because of the thoroughness with which the Alps have been explored and studied by botanists. Such a work is hardly yet possible in any portion of our country.

The supplement, which is a résumé of the work of recent students, adds nothing of general importance, but merely fills out details here and there. It would seem that in view of the great advance that has been made in the study of plant geography during the thirty years since the appearance of the first edition, a better plan would have been to rewrite the whole.—WILLIAM S. COOPER.

MINOR NOTICES

Local tree floras.—RAMALEY has published a thin volume[4] whose really useful part consists of descriptions of the trees of Colorado, with analytic keys and a considerable number of illustrations. These will be helpful to those who wish to study the woody plants. This part is prefaced by an essay of some 30 pages on the "wild flowers" of the state, with many halftones of vegetation and scenery, and a number of good outline drawings. The title, *Wild flowers and trees of Colorado*, is thus literally justified, but practically it is misleading. We hoped from the announcement to see a popular flora embracing the commoner

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[3] CHRIST, H., La flore de la Suisse et ses origines. pp. xiv+571+119. *pls.* 4. *maps* 5. Bâle-Geneva-Lyons: Georg & Cie. 1907.

[4] RAMALEY, F., Wild flowers and trees of Colorado. 8vo. pp. vi+78. *figs.* 70. Boulder, Colo.: A. A. Greenman. 1909.

plants of the state, after the fashion of some of the excellent books on the eastern flora. The demand for this sort of a book, we hope, will induce Dr. RAMALEY to prepare such a volume. The tourist travel alone would doubtless quickly absorb a reasonable edition.

MACKENSEN, a teacher in the San Antonio (Texas) High School, has published, as well as written, a handy a little pamphlet[5] on "all the woody plants growing naturally within five or six miles of the center of the city of San Antonio." According to the author's investigations there are just one hundred such species, of which at least ten are introduced. This number of species is surprisingly large, but is due to the author's lax conception of woody plants, for we find included various vines, agave, and the cactuses, not to mention a herbaceous mallow described as "woody below." The descriptions are untechnical, and there are some interesting observations on certain species. The lack of a key will limit the usefulness of the pamphlet.—C. R. B.

Asiatic palms.—The present volume, a monographic presentation of the species of Calamus,[6] records the results obtained by a careful specialist after years of study of living plants in their native habitat and the critical examination and comparison of dried specimens from the larger herbaria of the world. The subject-matter is arranged under ten headings, the first being an introductory essay of forty-five pages giving a detailed discussion of all parts of the plants entering in any way into their classification, the treatment of the genus by former authors, present limitations, especially with reference to Daemonorops and Palmijuncus, and finally the geographical distribution of species.

The author then introduces the taxonomy by giving a systematic conspectus of the species, arranged in sixteen groups, based primarily on the presence or absence of leaf-cirri, and further on the characters of the leaf sheaths and inflorescence; the conspectus is followed by a synopsis of the species (brief characterizations of the 201 recognized species); and lastly their enumeration with bibliography, detailed descriptions, and copious notes. With the exception of five species, known only from RUMPH'S descriptions and figures, all of those treated in the text have been beautifully illustrated by natural-size phototype reproductions from the author's own negatives. A complete index to species and plates concludes the volume. The work is an important contribution to taxonomic literature.—J. M. GREENMAN.

A forcing process.—JOHANNSEN'S process of forcing plants by treatment with ether vapor has been of considerable service in the commercial production of unseasonable flowers. A much simpler and in every way more practicable

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[5] MACKENSEN, B., The trees and shrubs of San Antonio and vicinity: a handbook of the woody plants growing naturally in and about San Antonio, Texas. 12mo. paper. pp. 51. *pls. 12.* San Antonio: The author. 1909.

[6] BECCARI, ODOARDO, Asiatic palms—Lepidocaryeae. Part I. The species of Calamus. Ann. Roy. Bot. Gard. Calcutta **11**:1–518. *pls. 238.* 1908.

process is described by MOLISCH in a little pamphlet, entitled *Das Warmbad*.[7] The treatment consists merely in immersing the shoots of potted plants (by inversion) in water of 30–35° C. for 9–12 hours and then keeping them in a warm (25°), moist, dark chamber until the leaves begin to appear, when further development proceeds under ordinary greenhouse conditions. Lilacs and spiraeas treated to the warm bath about mid-November flowered before Christmas, and azaleas early in January; while untreated plants, under the same conditions, had at this time hardly started their buds. The exact duration and temperature of the bath for securing the best results vary with different species and races. The process is already in use and is likely to be extensively practiced.—C. R. B.

The flora of Korea.—The first part of this work[8] includes the families Ranunculaceae to Dipsaceae, and their sequence is essentially that of BENTHAM and HOOKER in the *Genera plantarum*. Concise dichotomous keys to the genera introduce each family containing more than one genus, and similar keys at the beginning of each genus precede the enumeration of species. Under the species a very full bibliography is given, as well as the citation of exsiccatae and the general distribution. The Japanese name is also given in many cases. Several new species and varieties are published, and the text is augmented by fifteen full-page clean-cut illustrations. An index to genera mentioned in the flora concludes the part.—J. M. GREENMAN.

North American Flora.—Volume XVII, Part I, of this work[9] contains the following groups: Typhaceae by P. WILSON; Sparganiaceae, Elodeaceae, and Hydrocharitaceae by P. A. RYDBERG; Zannichelliaceae, Zosteraceae, Cymodoraceae, Naiadaceae, and Lilaeaceae by N. TAYLOR; Scheuchzeriaceae by N. L. BRITTON; Alismaceae by J. K. SMALL; Butomaceae, Poaceae (pars) by G. V. NASH. New species are described in the following genera: Sparganium (2), Echinodorus (1), and Trachypogon (2). One new genus (*Machaerocarpus*) of the Alismaceae is proposed, being based on *Damasonium californicum* Torr.—J. M. GREENMAN.

Diseases of trees.—A bulletin, embodying the results of a number of years' investigation of some of the more important diseases of deciduous trees by VON SCHRENK and SPAULDING, has been issued by the national Bureau of Plant Industry.[10] The bulletin contains a large amount of information (there is unfortunately little available) on diseases due to environment, to wound fungi (far

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[7] MOLISCH, H., Das Warmbad als Mittel zum Treiben der Pflanzen. 8vo. pp. 38. *figs. 12*. Jena: Gustav Fischer. 1909. *M* 1.20.

[8] NAKAI, T., Flora Koreana. Pars Prima. Jour. Sci. Coll. **26**:1–304. *pls. 1–15*. 1909.

[9] North American Flora, Vol. XVII, Part I, pp. 1–98. New York Botanical Garden, 1909.

[10] SCHRENK, H. VON, AND SPAULDING, P., Diseases of deciduous forest trees. Bur. Pl. Ind. U. S. Dept. Agric. Bull. 149. pp. 85. *pls. 10. figs. 11*. 1909.

the largest category), and to miscellaneous parasites and saprophytes, with some discussion of the decay of structural timbers. The Polyporaceae are the greatest devastators of our forests. A useful bibliography is appended.—C. R. B.

Vegetationsbilder.—The third part of the seventh series of KARSTEN and SCHENCK's well-known work[11] presents six plates of the vegetation of the moors, *Bockser* (high plains with dry grasses and sedges), and forest of the northern *Schwarzwald*, with text, by OTTO FEUCHT; the fourth illustrates the sea strand, littoral, sublittoral, and submontane formations on the Dalmatian coast, with text by L. ADAMOVIC; while the fifth pictures the various curious plants characteristic of the Abyssinian highlands.—C. R. B.

NOTES FOR STUDENTS

Taxonomic notes.—O. BECCARI (Phil. Jour. Sci. **3**:339–342. 1908) has described 3 new species and 2 new varieties of ferns from the Philippine Islands.—R. C. BENEDICT (Bull. Torr. Bot. Club **36**:41–49. 1909) records 4 new hybrids in the genus Dryopteris from eastern America.—C. CHRISTENSEN (Rep. Nov. Sp. **6**:380, 381. 1909) records a new species of Dryopteris from Brazil.—A. COGNIAUX (*ibid.* 304–307) publishes 5 new species of orchids from Jamaica.—F. S. COLLINS (Rhodora **11**:17–20. *pl. 78.* 1909) has published 4 new species of the genus Cladophora, and (*ibid.* 23–26) under "Notes on Monostroma" records a new form of *Monostroma orbiculatum* from Massachusetts.—E. B. COPELAND (Phil. Jour. Sci. **3**:343–357. *pls. 1–8.* 1908), under the titles "New genera and species of Bornean ferns," and "New species of Cyathea," has published 20 new species and 2 new genera (*Macroglossum* and *Phanerosorus*).—L. A. DODE (Bull. Soc. Bot. Fr. IV. **8**:648–656. 1908) describes 12 new species and 3 new hybrids of trees and shrubs; these include a Robinia from Colorado and a Salix from New Jersey.—F. EICHLAM (Monats. Kakteenk. **19**:1–5. 1909) characterizes a new species and 4 varieties of Mamillaria from Guatemala, and (*ibid.* 22–25) describes a new species of Pereskiopsis from the same general region.—A. D. E. ELMER (Leafl. Phil. Bot. **2**:445–594. 1908–1909) has described 101 new species and 3 varieties of Philippine plants. A new genus (*Elmeria* Ridl.) of the Zingiberaceae is proposed, and a synopsis of the genus Rubus is given, in which the author recognizes 17 species for the Philippine Islands, 3 being new to science.—A. ENGLER (Bot. Jahrb. **43**:161–198. 1909) has published new species of African plants as follows: 10 in the Olacaceae, 11 in the Opiliaceae, 2 in the Octoknemataceae, 11 in the Icacinaceae, and 13 in the Aizoaceae.—E. GILG (*ibid.* 97–128), in an article entitled "Balsaminaceae africanae," recognizes 85 species of Impatiens from Africa, and of these 26 are new to science.—M. GÜRKE (*ibid.* 199, 200) records 3 new species of Ebenaceae from Africa, and (Monats. Kakteenk. **19**: 12–14. 1909) describes *Rhipsalis Novaesii* and accompanies the description by illustrations of the flower; the plant is a native of Brazil.—W. HERTER (Bot.

[11] KARSTEN, G., AND SCHENCK, H., Vegetationsbilder. Series vii, parts 3–5. text and *pls. 13–30.* 4to. Jena: Gustav Fischer. 1909. *M* 4 per part.

Jahrb. **43**: Beibl. No. 98, pp. 1–56), under the title "Beiträge zur Kenntniss der Gattung Lycopodium," has published 48 new species, of which 30 are from the West Indies, Mexico, Central and South America.—J. D. HOOKER (Kew Bull. 1–12. 1909) presents the results of recent studies in the genus Impatiens from Indo-China and the Malayan Peninsula, recognizing for this region 27 species, of which 5 are new to science; a key precedes the enumeration of species.—M. A. HOWE (Bull. Torr. Bot. Club **36**:75–104. 1909) has published 7 new species of the Siphonales, and presents also a synoptical key to the living species of Neomeris.—F. KRÄNZLEIN (Rep. Nov. Sp. **6**:317. 1909) records a new species of Dendrobium from the Philippine Islands.—K. KRAUSE (Bot. Jahrb. **43**:129–160. 1909) has published 40 new species of Rubiaceae from Africa.—E. D. MERRILL (Phil. Journ. Sci. **3**:359–358. 1908) gives a synopsis of the Philippine species of Garcinia, recognizing 17 species, of which 5 are new; the same author (*ibid.* 369–382), under the title "Philippine Ericaceae," presents a consideration of the four genera representing this family in the Philippines, and accompanies Vaccinium (19 species) and Rhododendron (16 species) with determinative keys, characterizing 3 species and one variety as new; further, he gives (*ibid.* 385–442) a list of plants from the Batanes and Babuyanes Islands, describing 16 new species.—E. PAX (Bot. Jahrb. **43**:218–224. 1909), under the title "Euphorbiaceae africanae X," has published 15 species new to science, and proposes 2 new genera (*Holstia* and *Neopycnocoma*); the same author (*ibid.* 75–90) describes 43 species and 4 varieties of Euphorbiaceae as new to science.—J. PERKINS (*ibid.* 214–217) publishes jointly with E. GILG a new genus (*Ajrostyrax*) of the Styracaceae; the genus is represented by one species, *A. kamerunensis*, from Kamerun.—R. PILGER (*ibid.* 91–96), under the heading "Gramineae africanae VIII," describes 8 new species of grasses.—J. A. PURPUS (Monats. Kakteenk. **19**:38–41. 1909) describes and illustrates a new species of Cereus from Mexico.—B. L. ROBINSON and M. L. FERNALD (Rhodora **11**:33–61. 1909), under the title "Emendations of the seventh edition of Gray's Manual I," has given an annotated list of corrections, additions, extension of ranges, etc., and includes a new species of Callirhoë from Missouri.—R. A. ROLFE (Kew Bull. 61–66. 1909), in an article entitled "New Orchids: Decade 33," publishes 10 new species, of which 4 are American.—E. ROSENSTOCK (Rep. Nov. Sp. **6**:308–316. 1909) has published 9 species and 4 varieties of ferns as new to science; these are based on collections made by Dr. O. BUCHTIEN in Bolivia.—Different authors (*ibid.* 341–352), under the title "Ex herbario Hassleriano: Novitates paraguarienses I," have published 12 species and 16 varieties of flowering plants as new to science.—F. J. SEAVER (Mycologia **1**:41–76. *pls. 4, 5.* 1909) gives a synopsis of the Nectrieae of North America, recognizing 11 genera and about 40 species, 2 of which are here described for the first time; to the genus Nectria 20 species are referred, and a determinative key precedes their enumeration.—P. C. STANDLEY (Muhlenbergia **5**:46, 47. 1909) proposes a new species of Castilleja from Arizona.—W. WEINGART (Monats. Kakteenk. **19**:17–22. 1909) describes and illustrates a new species of Cereus from Paraguay.—E. O. WOOTON

and P. C. STANDLEY (Bull. Torr. Bot. Club **36**: 105–112. 1909) have described 11 species and one variety of flowering plants as new to science; these are based chiefly on the collections made in Mexico by O. B. METCALF.—J. M. GREENMAN.

Morphology of Monascus.—BARKER[12] and OLIVE[13] in their investigations of Monascus describe the ascogenous hyphae as arising from an oogonium, which has been fertilized by an antheridium, thus establishing a true sexual process for this form, while KUYPER[14] denies any fusion of sexual cells. IKENO[15] asserts that there are no ascogenous hyphae in Monascus, but that uninucleate cytoplasmic masses arise by free cell formation from the ascogonium, which in turn divide also by free cell formation to form the spores. KUYPER in the main agrees with IKENO as to the absence of sexual cells at the origin of the perithecium, while BARKER and OLIVE hold that the asci arise from the cells of the ascogenous hyphae, which arise from an ascogonium. DANGEARD,[16] however, regards BARKER'S antheridium as a nourishing cell for the ascogonium. According to DANGEARD the ascogonium divides into several cells, each of which gives rise to binucleate cells, which form the asci and whose nuclei fuse.

SCHIKORRA[17] has recently investigated pure cultures of two species of Monascus, *M. purpureus* Went. and an undetermined species, and believes that he has explained the inconsistencies of the above-mentioned authors. The life-history of both these species is similar. The female organ arises as a multinucleate subterminal cell of a hypha. This cell later divides into a trichogyne and oogonium. The apical cell is a multinucleate antheridium and fuses with the trichogyne, into which the male nuclei pass by means of a pore. There is an evident gap in observations at this point and the author next describes the male and female nuclei as arranged in pairs in the ascogonium. From the hypha below the sexual organs investing hyphae arise, which form a two-layered perithecial wall. Ascogenous hyphae originate from the fertilized oogonium. These hyphae contain nuclei in pairs, which multiply by conjugate divisions, although no such divisions are figured. The asci are formed from the subterminal cells

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[12] BARKER, P. T. B., The morphology and development of the ascocarp of Monascus. Annals of Botany **17**: 167–236. 1903.

[13] OLIVE, E. W., The morphology of *Monascus purpureus*. BOT. GAZETTE **39**: 50–60. 1905.

[14] KUYPER, H. P., Die Peritheciumentwicklung von *Monascus purpureus* Went. und *Monascus Barkeri* Dang., so wie die systematische Stellung dieser Pilze. Ann. Mycol. **3**: 32–81. 1905.

[15] IKENO, S., Ueber die Sporenbildung und systematische Stellung von *Monascus purpureus* Went. Ber. Deutsch. Bot. Gesells. **21**: 259–269. 1903.

[16] DANGEARD, P. A., Recherches sur développement du périthèce chez les Ascomycètes. Le Botaniste **10**: 177, etc. 1907.

[17] SCHIKORRA, W., Ueber die Entwicklungsgeschichte von Monascus. Zeits. Bot. **1**: 379–410. 1909.

of the recurved tips of these ascogenous hyphae. The ascus contains two nuclei, which fuse, and this fusion nucleus divides by a triple division to form the eight nuclei of the ascospores. The two ascus nuclei are looked upon as male and female, and their fusion completes the union of the gametes which initiated the development of the perithecium. The method of spore formation was not made out. The ascus walls finally degenerate, leaving the spores free in the perithecium.

From his studies SCHIKORRA concludes that Monascus is a true ascomycete, belonging to the family Aspergillaceae, in the order Plectascineae. It is placed here on account of the similarity of its perithecium to that of Penicillium and Aspergillus.

The presence of a true sexual process in the Ascomycetes has been established beyond a doubt in a considerable number of forms. The investigations of several species have shown, however, that a normal sexual process is not to be expected in all. The apparent absence of any nuclear fusion in the fertilized oogonium, although the sexual nuclei become arranged in pairs, brings Monascus into harmony with CLAUSSEN'S results on Pyronema, in which nuclear fusion is claimed to occur only in the ascus, a sort of modified sexual process, therefore, being present. Realizing how difficult it is observe nuclear fusion in the oogonium, on account of the small size of the nuclei and the apparent rapidity of the process, one cannot but feel that the actual fusion in the ascogonium may have been overlooked. CLAUSSEN'S and SCHIKORRA'S results support somewhat MAIRE'S contention that there is a tendency in the Ascomycetes to form a synkaryophyte analogous to that of the Basidiomycetes.—J. B. OVERTON.

Electricity and photosynthesis.—KOLTONSKI publishes an elaborate report[18] of experiments on the effects of electric currents of varying strength, density, and direction upon the number of gas bubbles given off by Elodea and Ceratophyllum in unit time. The work is an extension of that of THOUVENIN,[19] and proceeds, like his, upon the assumption (which may usually be valid) that under normal conditions the number of these bubbles constitutes a fair measure of the rate of photosynthesis; but it is hardly necessary to say that the action of an electric current may create conditions which render this assumption invalid. It is interesting to know that electric currents produce an effect upon the evolution of gases in these plants; but it is impossible to interpret KOLTONSKI'S results. He presents them, indeed, as only a starting-point for further investigations.—C. R. B.

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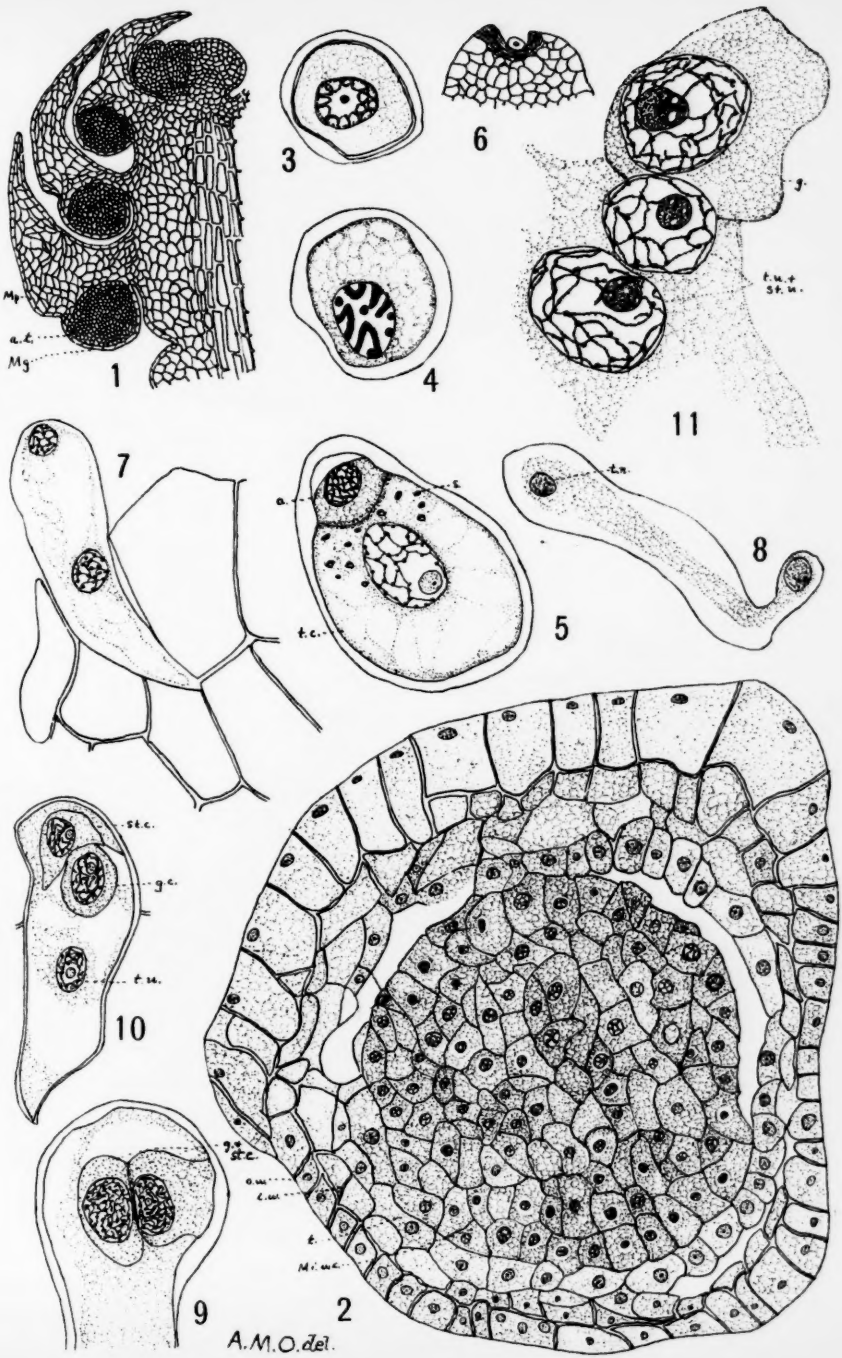
[8] KOLTONSKI, A., Ueber den Einfluss der elektrischen Ströme auf die Kohlensäureassimilation der Wasserpflanzen. Beih. Bot. Centralbl. **23**: 204–271. *figs.* 4. 1909.

[9] THOUVENIN, M., De l'influence des courants électriques continus sur la décomposition de l'acide carbonique chez les végétaux aquatiques. Rev. Gén. Bot. **8**: 433–450. 1896.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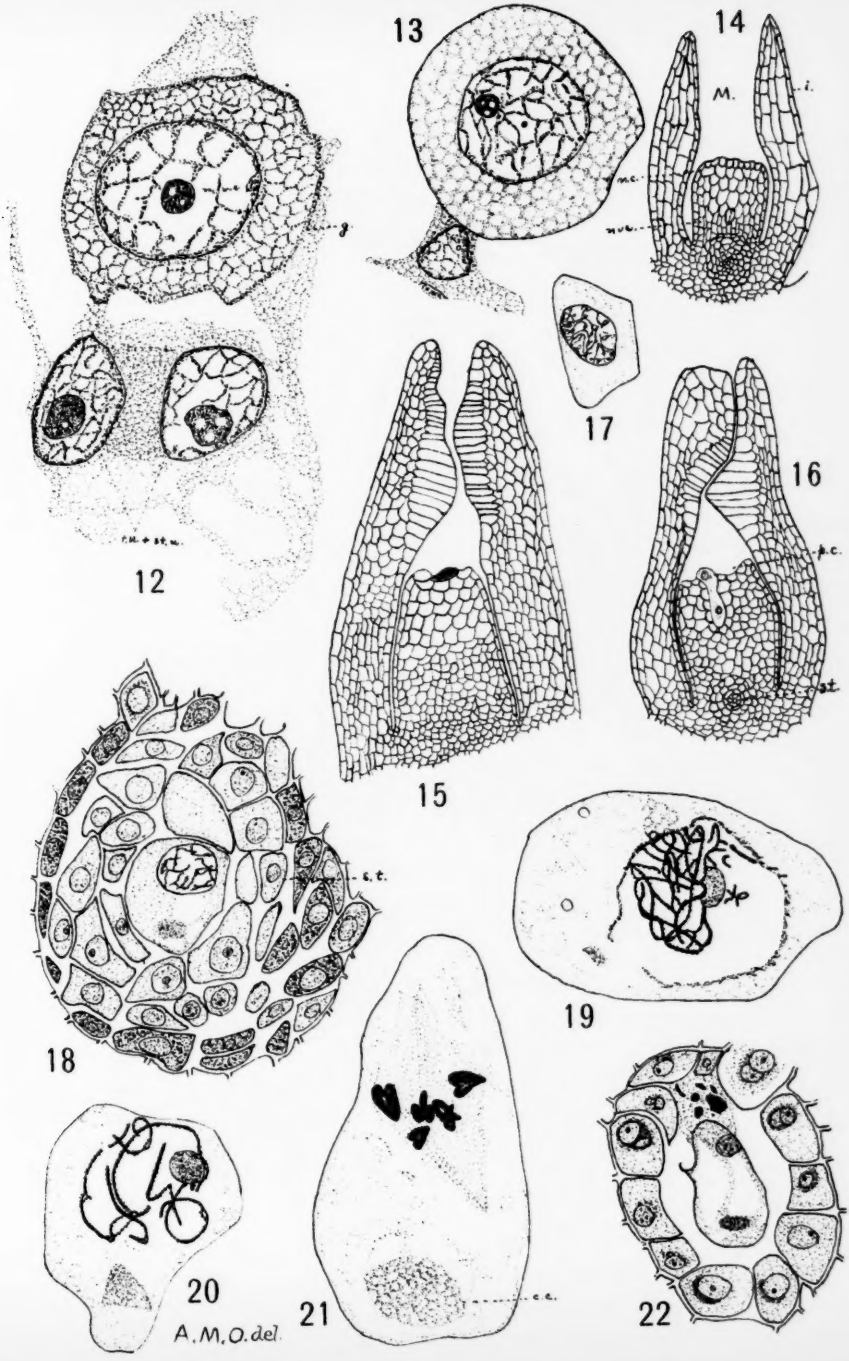
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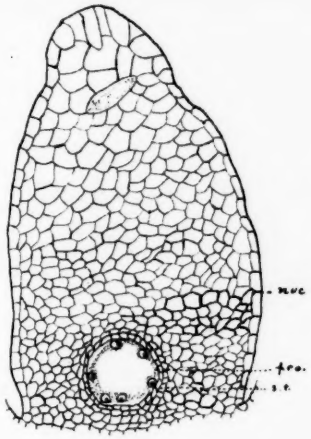

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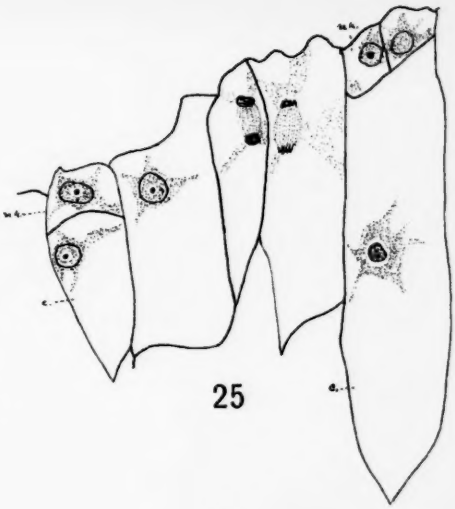

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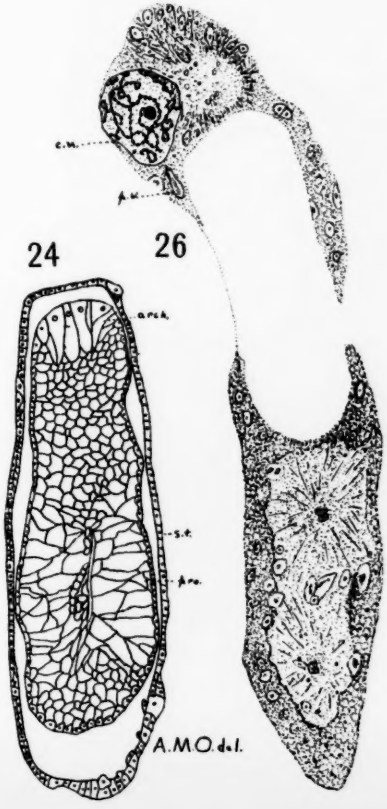

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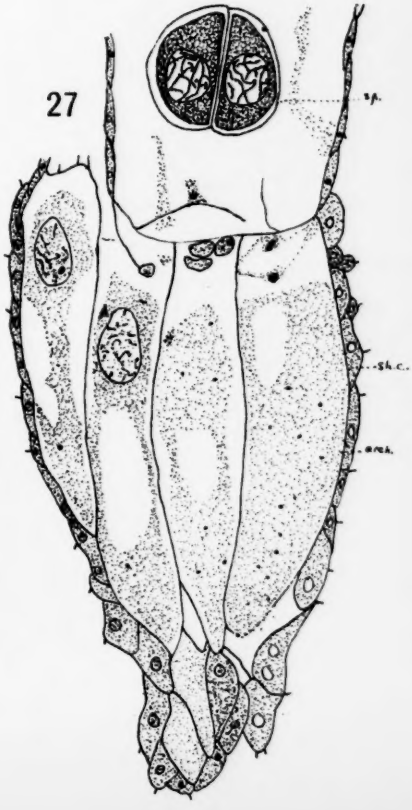

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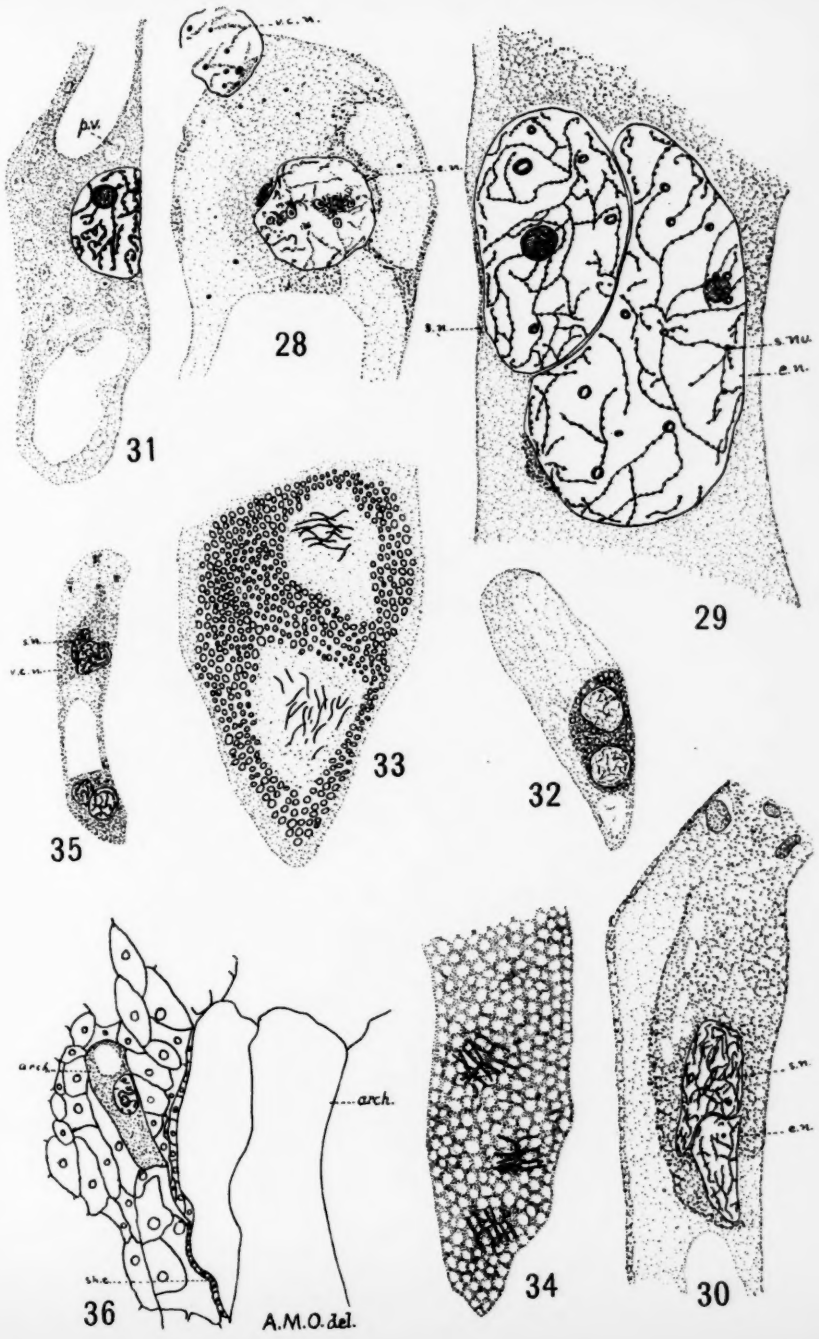
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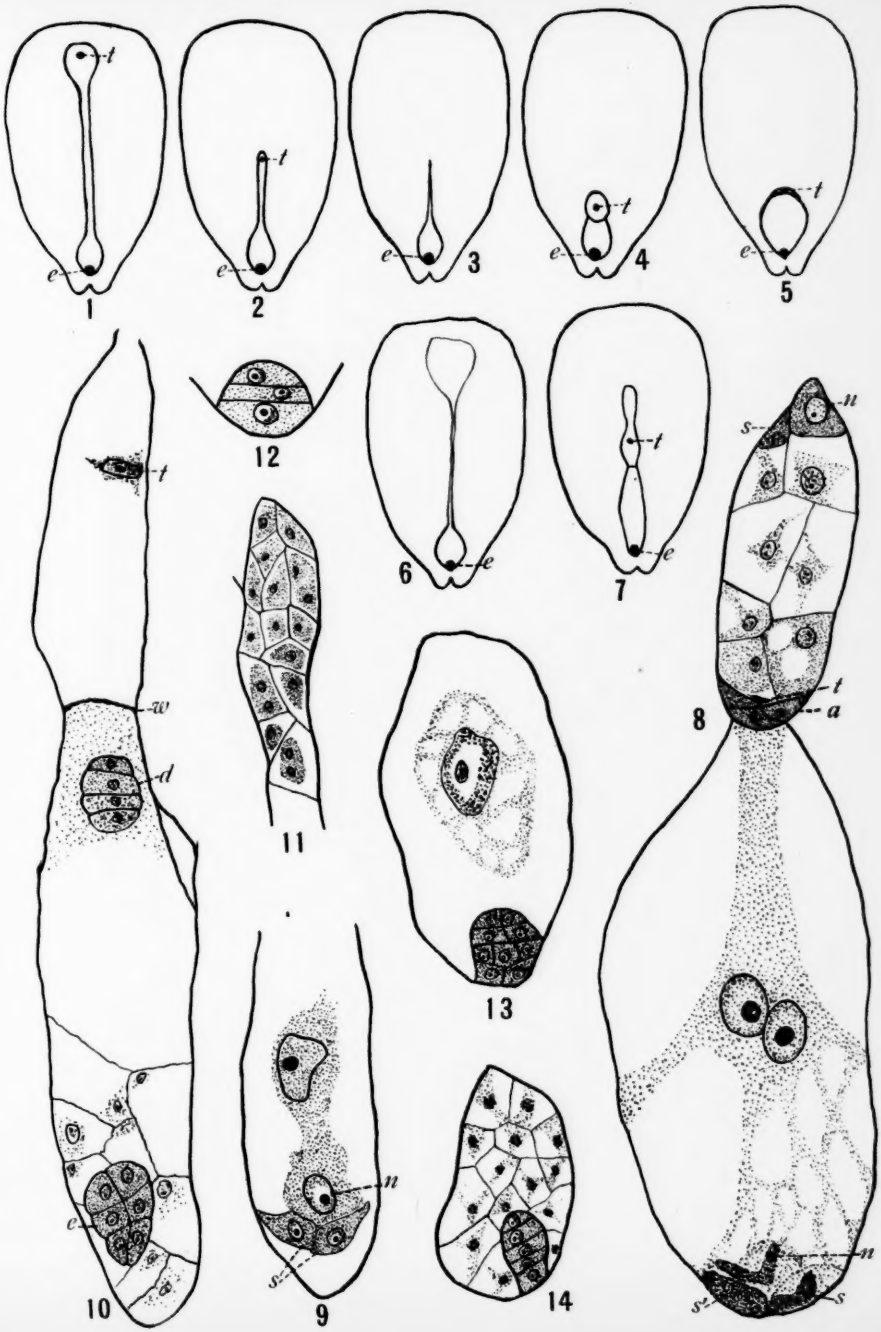
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